Gerhard Billek
Hamburg, Germany

Health aspects of thermoxidized oils and fats

Early studies used extremely overheated fats which were toxic if fed to animals. Later fats and oils were heated in equipments for deep-fat frying under the conditions of good commercial practice. Such heated fats showed no detrimental effects even if fed in long-term feeding trials. The results are reported and critically evaluated. However, certain fractions of the heated fats, e.g. the so called “Total Polar Materials” caused clearly noticeable effects in the feeding studies: growth retardation, increased liver and kidney weights, and disorders of the enzyme system but only if fed in high doses. In the last years several research groups specialized in the use of cell cultures and enzymological methods and gained insight into resorption and metabolism. Model compounds or compound mixtures have been synthesized. Their structure and/or composition deviated more or less from the genuine compounds or fractions of the heated fats and the results of these studies must not be generalized.

New analytical procedures allow to isolate well-defined fractions, and quantitative data about their contents in heated fats are obtained: (a) harmless mono- and diglycerides and free fatty acids, (b) dimeric and polymeric triglycerides and dimeric fatty acids, harmless as well because of very low resorption rates and (c) oxidized triglyceride monomers. Their saponification and degradation products are oxidized monomeric and dimeric acids, oxidized cyclic fatty acids, and other polar compounds. These low molecular oxidized compounds are nutritionally suspect.

Since 60 years and with considerable effort more or less systematic investigations have been performed in many countries to answer the question, if heated fats are detrimental to health.

Keywords: Frying fats and oils, polar materials, cyclic dimeric fatty acids, hydroperoxides, malabsorption, toxicity, detoxification.

1 Overheated fats

In the year 1938 Roffo [1] heated various fats and oils at 250–350 °C and fed them to 1000 rats. He claimed that carcinomas of the stomach were produced. His results have never been confirmed. However, since that time the general opinion was prevailing that heated fats can be detrimental to health. Although his heating conditions have been criticized as being quite unrealistic, other researchers continued with experiments using again extremely high temperatures or long heating periods.

Extensive studies on the toxicity of heated oils have been carried out by Crampton et al. [2]. A series of unsaturated oils, like soybean, corn, cottonseed, and other oils, have been heated at 250–300 °C for 6–24 h under exclusion of air. When the heated oils and fractions therefrom were fed to rats at 20% in the diet, they caused loss of weight and high mortality. The toxicity lay in the distillable fraction of the urea non-adduct of the fatty acids. The polymer fraction caused diarrhea but did not appear to be highly toxic.

Firestone et al. [3] heated cottonseed oil at 225 °C for 19 h. When these samples were fed to rats the animals lost the ability to absorb these oils and the authors argued that such oils even could interfere with absorption of other nutrients. The urea non-adduct of these oils were fed to mice and caused their death.

These frightening effects aroused much speculation. However, they originated from the great variety of uncommon compounds formed under the extreme heating conditions [4, 5]. Such work is unrealistic since severely damaged fats are not consumed with the human diet. Nevertheless, these experiments have not been useless. The chief value of work with badly abused fats and oils has been in stimulating work with fats heated under household conditions or under good commercial practice. Furthermore, they guided researchers as to the kind of effects they should look for if experimenting with oils having a lower degree of deterioration.

2 Commercially used oils and fats

In one of the most significant long-term feeding trials, performed by the well-known German nutritionist Konrad Lang [6], soybean oil and hardened groundnut oil were heated in a commercial fryer with and without frying
goods at 175 °C. Heating time was 8 h per day. 10 days were necessary until the amount of Total Polar Materials (TPMs) in the oils reached a value of 20–25%. It should be mentioned here that the value of 24–25% of TPMs is an important borderline in the assessment of frying oil quality [7]. This means that the fats and oils used in this study have not been deteriorated according to recommendations of health authorities. The unheated and the heated oils were fed to three generations of rats at 10% in the diet. It has to be mentioned that this study took 10 years to complete. Even after such a long time there was no difference to be observed between feeding the unheated and the heated oils. Of the many parameters investigated in this study only one is shown here: The death rates after feeding the unheated and the heated soybean oils (Fig. 1). The only significant difference is that the animals in the second generation lived somewhat longer.

This study, although finished more than 20 years ago, has never been criticized and the conclusions are still valid: “Frying fats heated under the conditions of a good commercial practice are not detrimental to the health of the test animals” [6]. Similar but much shorter studies were conducted by other research groups with rats [8, 9] and dogs [10] and practically yielded the same results.

However, in the last decades other reports have been published on experiments with frying fats, which had not been abused, that is to say, they were still of a good quality. Surprisingly they showed harmful effects, like diarrhea, weight loss, and sometimes even the sudden death of the test animals. In most cases the reasons for these pitfalls were obvious: Imbalanced diets with a lack in essential fatty acids and vitamin E [5], fats and oils as the sole nutrient [11], oral dosing of large amounts, e.g. with a stomach tube [12], determination of lethal dose LD50 of fats and oils [13]. One should be very careful in evaluating such experiments and in reporting their results.

In the 10-years study of Lang et al. [6] practically no effects could be observed; the “no-effect level” was not surpassed. It was not possible to propose any amount of an “acceptable daily intake”. A few years later, we – in the Unilever Research Laboratory in Hamburg – started another approach to solve this problem [14].

3 Feeding of the Total Polar Materials

Gertz [7] reported on a very simple method to isolate the so-called TPMs from heated fats. Using a silica gel column and two different solvents it is possible to separate the heated oil into two fractions:

– the non-polar fraction containing all the material which has not been oxidized during the heating procedure and
– the TPMs, the amount of which is regarded as being a good measure for the deterioration of heated fat.

In his 10-years experiment Lang [6] used the heated oil and he did not see any indication of a possible harmfulness. It was our idea to repeat a similar long-term feeding experiment but to use the Total Polar Materials alone, the fraction in which all the oxidized compounds are concentrated [14]. Several tons of sunflower oil which was used for the production of fish fingers in an industrial fryer were taken at the end of a production period when the oil usually was discarded according to the practice of this company. The oil was fractionated in a stainless steel column with a volume of 180 l, which was fed with 100 kg of silica gel. The solvent systems were not the same as in the analytical procedure [7]. We used disisopropyl ether instead of the common diethyl ether for safety reasons and isopropanol for better recovery of the second fraction.

Fig. 2 shows the material and the fractions used. The fresh sunflower oil (F) was used for the control group of the animals. The heated oil (H) after production of the fish fingers was fractionated on the silicagel column to obtain the non-polar (N) and the polar fraction (P). Each fat or fat fraction was fed to rats at 20% by weight in the diet over a period of one and a half year. Each group consisted of 60 animals; they were kept in single cages and the diet was given ad libitum [14].

Fig. 3 shows the mean weights of the male animals. The highest increase in weight was observed with the fresh sunflower oil (F). The group receiving the polar fraction (P) showed a significantly lower gain in weight. With the female animals (not shown here) the difference between the groups was somewhat smaller but in the same order. The weights of the livers and kidneys were higher in the
groups receiving the polar fraction, which is an indication for some effects upon these organs.

Many parameters of clinical chemistry were checked after 3, 6, 12, and 18 months. Only the activities of the serum enzymes glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were approximately 20% higher in the groups receiving the polar fraction. All other parameters measured, however, were normal and were the same in all groups. Moreover, the histological investigations revealed no changes in any tissues of the test animals indicating that the animals would have recovered, if they had been returned to a normal diet [14].

Some years later Grandgirard et al. [15] looked upon the curves on Fig. 3 and made the following remarks: It is reasonable that the polar fraction (P) shows the lowest gain in weight if compared with the fresh oil (F). Again it is reasonable that the heated oil (H) shows some effect upon gain in weight although a somewhat smaller one. But why is there a difference between the fresh oil F and the non-polar fraction (N)?

Grandgirard et al. [15] repeated our sample preparation (Fig. 2) and looked for the presence of cyclic fatty acid monomers. They found up to 0.4% in the heated oil (H) but only up to 0.2% cyclic monomers in the corresponding polar fractions (P). They concluded that the rest must have been in the non-polar fraction (N) and must have been responsible for the lower gain in weight of the animals. Their explanation, why the cyclic monomers show up in both fractions is very plausible. Triglycerides with a cyclic monomeric fatty acid in the molecule are non-polar, but if they contain some additional oxygen functions, they are polar and, therefore, to be found in the polar fraction. Since we had no samples left, we could not look for the presence of cyclic monomers in the fractions we used. But we accept the explanation of our French colleagues. This leads to the question: How dangerous are cyclic fatty acid monomers?

4 Cyclic fatty acid monomers (CFAM)

A study of considerable importance has been performed by Artman et al. [16] of Procter & Gamble. They heated various oils under normal conditions and isolated a certain fraction, the distillable part of those fatty acids which could not be adducted by urea. This fraction contained anything except straight chain fatty acids and, of course, contained cyclic fatty acid monomers (CFAM). That fraction proved to be somewhat toxic when fed to animals in high doses. That result was a matter of concern since this fraction was isolated from normally heated fats and oils.

The authors isolated more than 130 compounds and identified many of them. They explained that it was the purpose of their work to look for undesirable compounds which might be present in the heated fats. Despite their efforts they found not even one which showed unexpected structures. They summarize their work as follows: “Actual used frying fats contain only very small quantities of substances which are toxic only when administered in large doses to weanling rats, and that the fats themselves produce no appreciable ill effects on animals consuming them”.

Starting from unsaturated fatty acids CFAM can be synthesized in good yields. Such a product was prepared by Perkins et al. [17] to study the toxicity of CFAM. They heated methyl linolenate with NaOH in ethylene glycol at 195 °C for 1 h under nitrogen. A mixture of cyclohexa-
dienoic compounds with approximately 10% aromatic compounds was obtained. These CFAM were given in amounts of 0.0075–0.15% in diets with different levels of proteins (8–15%). At the same protein levels there was practically no difference in weight increase, food efficiency, or organ weight of the test animals. Only the lipid content of the livers of the rats receiving the 0.15% dose was increased. In these experiments the CFAM with a 6-membered ring showed an unexpectedly low toxicity. Addition of proteins to the diet alleviated the toxic symptoms [17].

CFAM, uniformly labelled with $^{14}$C, were prepared according to the same procedure and given in the same doses to rats by oral dosing. After two days about 40% of the radioactivity has been excreted in the urine and only about 14% appeared in the CO$_2$. This accounts for the radioactivity has been excreted in the urine and only 35% in the CO$_2$. The low toxicity of the CFAM can be explained by the rapid excretion of these compounds [17].

Depending on the fatty acids from which they originate, CFAM isolated from heated frying oils show very many different structures [18]. Perkins et al. [17] investigated only one, a 6-membered ring type prepared from linseed oil, which is not a commonly used frying oil. Furthermore, his synthetic sample was not oxidized whereas in heated frying oils at least part of the CFAM carry oxygen functions.

The next step to solve the CFAM problem was to isolate them from normally heated frying oils. For this purpose excellent analytical methods [19] can be used which already gave us detailed information about the structures of CFAM and their amounts in used frying oils depending on the source and heating procedures. In experimentally heated and even somewhat overheated oils not more than 0.6% (Tab. 1) and in ill-treated oils from snack bars in various countries not more than 0.7% were found (Tab. 2). This means that it is not possible to isolate sufficient material for animal feeding experiments. We do not know how much oil was used in Konrad Lang’s 10-years experiment, but in the Unilever Research Laboratory in Hamburg 300 kg of polar material have been necessary for a 1$^{1/2}$-year study with 240 rats. Fortunately there are other possibilities to test toxicity.

Hydrogenated soybean oil [27] was used for the production of fish fingers. The cyclic monomers, a mixture of 5- and 6-membered ring compounds have been isolated and fed to rats. Several liver enzyme activities have been measured. After feeding the cyclic monomers a significant increase of the Cytochrom P-450 reductase was observed. This enzyme is responsible for a certain detoxification mechanism and its activity increases if foreign compounds, e.g. drugs, enter the mitochondria. This enzyme system is well-known for many years; already Konrad Lang looked upon it when working with heated fats. Other enzyme activities in the liver decrease after feeding the cyclic monomers, e.g. carnitine palmitoyl transferase, which means less degradation of fatty acids. The activity of the isocitrate dehydrogenase was decreased as well, with the effect of an impairment in the Tri-carboxylic Acid (TCA) Cycle.

### 5 Dimeric fatty acids

Already in the 1970s dimeric fatty acids have been successfully used as the first indicator for heat treatment of fats and oils [28]. The higher the temperature applied or the longer the reaction time the more could be detected. Already at that time we have been interested if these substances might be detrimental to health and we made a few experiments with labeled compounds.

We prepared the methyl esters of fatty acids from safflower oil [29], heated them to 280°C and isolated the dimers. When fed to rats only 1% of the substance was metabolized to CO$_2$ but most of the radioactivity (80%) was excreted with faeces or found in the gastrointestinal tract. Obviously only a very small part of the dimers has been metabolized.

A few years later Perkins et al. [30] made a similar experiment again with labeled dimers and came to the same result. He found 85% in the faeces and less than 6% in the CO$_2$. He made feeding experiments with 0.1, 1.0, and

### Table 1. Cyclic fatty acids in simulated frying tests.

<table>
<thead>
<tr>
<th>Oil</th>
<th>Goods</th>
<th>Time</th>
<th>Amount [%]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>Potatoes → 100 h</td>
<td>0.33–0.57</td>
<td>J. B. Meltzer et al. [20]</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>None → 216 h</td>
<td>&lt; 0.2</td>
<td>Pei-fen Wu et al. [21]</td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td>Various 80 h</td>
<td>0.07–0.18</td>
<td>J. A. Rojo et al. [22]</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Cyclic fatty acids in commercial frying oils.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Samples</th>
<th>Amounts [%]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>25</td>
<td>0.02–0.50</td>
<td>E. N. Frankel et al. [23]</td>
</tr>
<tr>
<td>Egypt,</td>
<td>9</td>
<td>0.17–0.66</td>
<td>E. N. Frankel et al. [23]</td>
</tr>
<tr>
<td>Israel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hungary</td>
<td>8</td>
<td>0.02–0.16</td>
<td>A. Gere et al. [24]</td>
</tr>
<tr>
<td>France</td>
<td>31</td>
<td>0.01–0.25</td>
<td>J.-L. Sébédio et al. [25]</td>
</tr>
<tr>
<td>France</td>
<td>21</td>
<td>0.01–0.09</td>
<td>G. Poumeyrol [26]</td>
</tr>
</tbody>
</table>
5.0% dimeric fatty acids in the diet and found no difference in food consumption, weight increase, and liver weights. Some troubles started only at the 20% level because of malabsorption. The authors concluded that dimeric fatty acids are not toxic. In our experiments we fed methyl esters. Perkins fed free fatty acids, but originally the dimeric fatty acids have been part of the dimeric triglycerides and these compounds are in the diets.

6 Dimeric triglycerides

We have been interested in looking for harmful compounds formed during high temperature deodorization of soybean and other oils. Dimeric triglycerides in heated oils can be detected and isolated by means of size exclusion chromatography without any difficulty. Since oxygen is excluded during deodorization non-polar dimeric triglycerides are obtained. Their presence in fats and oils is a good indicator of a preceding temperature treatment [31].

For animal experiments we isolated 20 kg of this product and fed it to mice over a period of 1 year [32]. We had not enough material to feed rats. Since there was no difference in the gain in weight between the test animals and the controls we are sure that the lipases worked well and the intact fatty acids were well absorbed. The dimeric fatty acids were excreted with faeces at a high percentage. With an LD50 of more than 20 ml/kg the dimeric triglycerides are not toxic.

7 Triglyceride mono-hydroperoxides

The first step in the oxidation of fats and oils is the formation of hydroperoxides, which are unstable in hot oil, but they can be formed again in periods of cooling the oil and during storage of the fried food. They show very low absorption in the organisms, but they are hydrolyzed by lipases as fast as the original triglycerides. All low molecular hydroperoxides are very toxic, if they are injected into the veins, but rather harmless if they are fed via the gastrointestinal lumen. Here they are converted to hydroxy acids by enzymatic action of a peroxidase. These hydroxy acids are rather harmless. If hydroperoxides are eaten, how much this is we do not know exactly, they disappear in the gut.

8 Secondary products (low-molecular weight)

At frying temperatures hydroperoxides decompose faster than they are formed. The peroxide value of a hot frying bath is zero. They disappear with the formation of secondary products. Cleavage occurs either on the one or on the other side of the alkoxy radicals (Fig. 4). Two types of aldehydes are formed [33]:

1. A series of aliphatic aldehydes of low molecular weight. Depending on the structure of the radical quite a lot of different compounds with a lower molecular weight as the original fatty acid are formed: aldehydes, ketones, epoxides, hydrocarbons, and others. Most of them are volatile at frying oil temperatures and evaporate from the heated oil. Some are responsible for the flavor of the fried goods.

2. Another group of aldehydes remain attached to the triglyceride molecule and is retained in the fried food. A typical example for this kind of substances is 9-oxononanoic acid. Again a series of similar compounds have been detected. Quite a lot of them have been synthesized and their properties could be tested [34]. All of them showed a more or less high toxicity. They are cytotoxic, hepatotoxic, carcinogenic, mutagenic, and more although their bioavailability is not always known. They are the only concern we have with abused frying fats and oils. Many questions are still open and further research will be necessary.

9 Polymer material

Márquez-Ruiz et al. [35] used enzymatic hydrolysis and a very short experiment gave impressive results (Fig. 5). Heated olive oil and the polar material isolated from this oil were hydrolyzed with pancreas lipase. The triglycerides in the heated oil disappeared after 2 min, but the polymer material remained practically untouched. Dimeric triglycerides were partly hydrolyzed as well as trimeric triglycerides but to a smaller degree. Hydrolysis with pancreas lipase is more difficult as the molecular weight in-
creases. Similar results were obtained with the polar material. The oxidized triglycerides are hydrolyzed very fast, but the polymers again remain intact.

With this technique another remarkable result was obtained. Hydrolysis of intact triglycerides can be affected by the presence of dimers and polymers in an abused frying oil, in other words an oil is digested slower, if it contains alteration products [36].

10 Conclusions

Even without the influence of oxygen unsaturated fatty acids polymerize at frying temperatures. New bonds are formed between the fat molecules leading to dimeric, trimeric, and higher polymeric triglycerides. The intact fatty acids of the new molecules are well metabolized, only the higher polymers withstand enzymatic cleavage.

Dimeric fatty acids are well absorbed but degraded in the organisms only to a small degree, i.e. only as long as β-oxidation works. The remaining torso is excreted with the urine by some kind of a detoxification mechanism. Dimeric and higher polymeric triglycerides are not toxic as well as their saponification products.

New bonds within a fatty acid create series of compounds with different cyclic structures. Fractions isolated from heated frying fats containing such compounds have been toxic when fed to test animals. However, later experiments, with well-defined mixtures of cyclic fatty acid monomers, revealed that they may interfere with the functions of metabolic enzymes in the body, but the reports of high toxicity could not be confirmed. Probably oxygen containing compounds of unknown structure have been responsible for the observed toxicity.

Oxygen from the air and unsaturated fatty acids in the oil, initiated by free radicals, start chain reactions, and hydroperoxides accumulate in the oils. Triglyceride monohydroperoxides are highly toxic after parenteral application to test animals.

They are rather harmless if fed via the alimentary canal being detoxified by means of the omnipresent peroxidases. At frying bath temperatures, however, the hydroperoxides decompose faster then they are regenerated. Cleavage of the fatty acid near the site of the hydroperoxide radical leads to two series of different compounds (Fig. 4). On the one hand are the harmless volatile aliphatic aldehydes evaporating from the frying bath. On the other hand, we have oxygen bearing fatty acid fragments still attached to the triglyceride molecule, remaining in the bath and migrating into the food. The latter group of oxidized compounds, many of them of unknown structure, could be detrimental to health and further research in this field seems to be necessary.

References

Health aspects of thermoxidized oils and fats


[Received: June 8, 2000; accepted: July 24, 2000]