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Trans-Diene and Triene Fatty Acids in Edible Oils from Deodorization

Key words: Trans-diene and triene fatty acids, Isomerized fatty acids,
Deodorization of edible oils

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Introduction

Another paper on *trans* fatty acids? During more than two decades, there have been exhausting discussions on possible negative effects on health of *trans*- and positional isomers of oleic acid, present at high concentrations in partially hydrogenated oils and fats (margarine and fats for food industry). Still no generally accepted conclusion could be found. Isomerization of oleic acid also occurs during strong bleaching of oils, although to a minor extent only. This paper does not deal with these *trans*-monoenes, but with the *trans* isomers of linoleic and linolenic acid, i.e. of essential fatty acids («*trans* isomers» meaning all acids with at least one double bond in *trans*). *Trans*-polyenes easily escape notice in the shade of the *trans*-monoenes, also because they became important only recently. *Trans*-dienes and trienes are formed at temperatures above 200 °C (1–5), thus primarily during deodorization, and are, therefore, a problem for all refined edible fats or oils. *Cis* double bonds are converted into *trans*, but there is virtually no shift in the position of the double bond.

Diene and triene fatty acids belong to the essential fatty acids, needed as educts for the synthesis of a variety of compounds (such as prostaglandins), and should, therefore, be of special concern. According to the current assumption that a *trans* double bond is not recognized by enzymes (e.g. *trans*-monoene acids seem to be metabolized as the corresponding saturated acids), the trienes with one double bond in *trans* should be of particular concern, because they might be confused with a diene. Δ15 *trans*-linolenic acid would be mistaken as linoleic acid (6). Exactly these *trans*-trienes, however, are formed at high rates during deodorization at high temperature.

The subject of the *trans*-dienes and trienes requires immediate attention because of the present trend to perform deodorization at massively increased temperatures. The most important reason for this is the on-going replacement of chemical by physical neutralization: free fatty acids are no longer removed by alkali, but by evaporation. This procedure is superior concerning environmental and economic aspects, but is also responsible for the present problem of the *trans*-dienes and trienes, since temperatures exceeding 230 °C are required. Higher temperatures also enable to abbreviate deodorization, i.e. to increase the output of the plant, and to substitute a better vacuum. Classical deodorization at 180–185 °C following chemical neutralization produced less than 0.1% *trans* isomers. Physical raffination increased isomerization by 1–2 orders of magnitude at least.

Transition to substantially higher deodorization temperatures was justified by ecological advantages; possible physiological drawbacks were played off by arguments such as that milk fat also contains high concentrations of *trans* fatty acids. However, the *trans* fatty acids found in milk and milk products are not the *trans*-dienes and trienes formed during high temperature deodorization (nor do they correspond to the many positional isomers created by hydrogenation). The same disqualifies the argument that large populations have consumed partially hydrogenated fats containing high concentrations of *trans*-monoenes over decades without apparent serious problems.

Little information is available about the fate of essential fatty acids with double bonds in *trans* and their effect. *Sébédio* et al. (6) have shown that Δ15 *trans*-linolenic acid can be metabolized by the Δ6-desaturase, though at a lower rate than the *cis* isomer, i.e. that it inhibits the activity of the first enzyme of the desaturation-elongation procedure. The same *trans*-acid has been detected in phospholipids of the brain. This confirms the concern about a possible disturbance of metabolism, but far more information will be needed for a concluding evaluation of the effects on *trans*-polyenes.

There are numerous techniques for analyzing *trans* fatty acids, including infrared spectroscopy (7, 8), HPLC (9) and packed column GC (10). Mostly, however, capillary GC-FID is used with long columns coated with cyanopropyl polysiloxanes (11–13). Literature data on *trans* fatty acid intake usually refers to the sum of all *trans* fatty acids (14–17).

This paper reports data on the concentrations of the *trans*-dienes and trienes in oils from the Swiss market, as well as on the degree of isomerization. Laboratory experiments were performed in order to correlate this data with the conditions during the deodorization process. The primary intention is the promotion of a discussion: since conclusive toxicological studies are likely to take many years, opinons should be formed on to what extent such *trans*-polyenes should be tolerated in the mean time.

Experimental

Oils were transesterified to fatty acid methyl esters (FAME) at ambient temperature (18) and extracted into heptane. They were analyzed by on-column injection onto a 100 m x 0.25 mm i.d. separation column coated with SP-2560 (Supelco). Chromatographic conditions were periodically re-adjusted to provide sufficient separation between the acids 18:3 and 20:1 and finally involved 1.5 bar carrier gas inlet pressure (hydrogen), injection at 85 °C (1 min), and a temperature program by 20 °/min to 160 °C, by 2.5 °/min to 220 °C, and by 5 °/min to 235 °C. Peak areas were determined by ChromCard, using manual adjustment of the baseline where necessary. Concentrations were expressed as percent of the peak area summed up for all FAME. Figure 1 shows the GC-FID chromatogram of a soybean oil containing about 0.7% *trans*-dienes and 1.1% *trans*-trienes.

Commercial oils were obtained from the local market and involved bottled oils as well as oils in preserves and other products. Most of the bottled oil is from Swiss oil refineries, whereas the oils from preserves were all imported.

For laboratory experiments, oils were heated in flame-sealed glass tubes: Pasteur pipettes were flame-sealed on one end, filled 3 cm high with oil, evacuated during 3 min, and flame-sealed on the other end. These tubes were kept between two plugs of glass wool in a beaker and heated in a GC oven. Temperature was controlled by a thermocouple inserted in an oil sample the tube of which was left open at the top. Temperatures indicated were reached after 15 min and maintained for 45 min., 120 min., 240 min, or 360 min. Experiments were performed with raw and bleached rapeseed oil, as well as with cold pressed soybean oil.

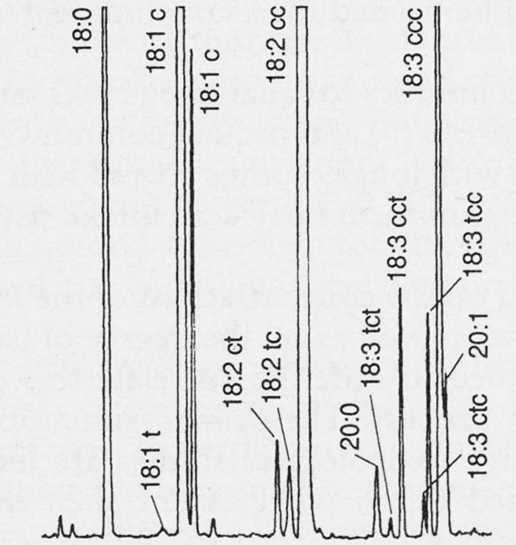

Fig. 1. The region of the C18 fatty acids separated on the cyanopropyl silicone stationary phase

Results

Oils from the market

Results on cis/*trans* isomerization of polyenic fatty acids can be presented in two ways: as concentrations or as degrees of isomerization. During deodorization, linolenic acid is isomerized some 14 times more rapidly than linoleic acid (see below) – compared to these two acids, cis/*trans* conversion of oleic acid is negligible. If the data is depicted as total concentrations of *trans*-polyenes, oils containing elevated concentrations of linolenic acid (rapeseed and soybean oil) tend, therefore, to look particularly alarming. Presentations as proportions of isomerized linoleic or linolenic acid will rank oils differently. As both options have their merits, results will be presented in both ways.

Concentrations of trans-dienes and trienes

As shown in figure 2 (left), bottled rapeseed and soybean oils from the Swiss market contained between 0.11 and 2.3% of summed *trans*-dienes and trienes (average of 1.2%). Half of all the samples contained less than 1% *trans* fatty acids, only few less than 0.5%. Among the 31 samples of bottled oils containing less than 1% of linolenic acid (such as sunflower, peanut, and olive oil, right in figure 2), only one exceeded 1% *trans*-polyene fatty acids. Half of all samples contained less than 0.3%, primarily of *trans*-dienes; the average of the *trans*-polyenes was 0.37%. Concentrations of *trans* fatty acids found in oils (mostly soybean or rapeseed oil) from preserves of sea foods (such as sardines, anchovies, and tuna), vegetables (artichokes, mushrooms, dried tomatoes, peppers) and sauces tended to be higher: they were slightly higher as an average (figure 3), but there were also extreme

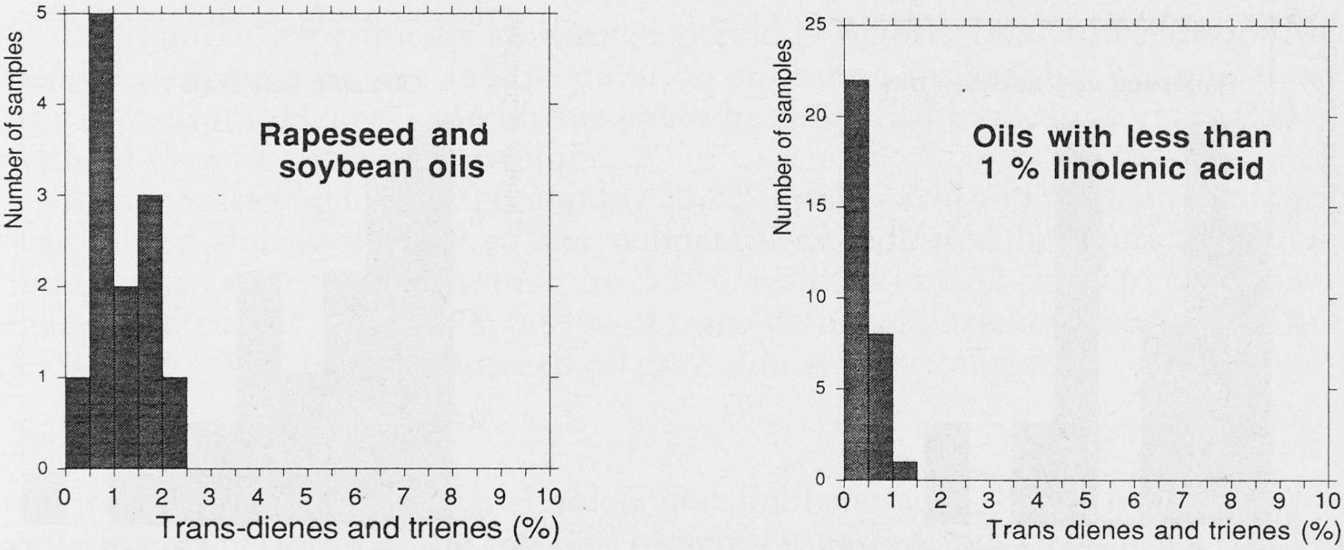

Fig. 2. Concentrations ((%) of the total of the fatty acids) of *trans*-dienes and trienes in soybean and rapeseed oils (left) as well as in oils containing less than 1% linolenic acid (right)

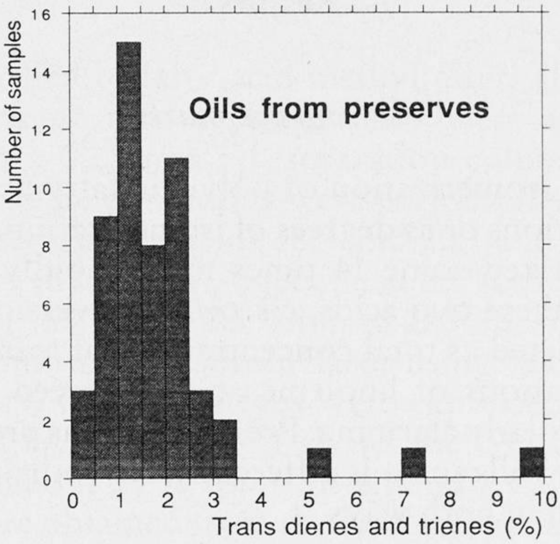

Fig. 3. Concentrations of *trans*-dienes and trienes in oils from preserves

samples. The highest result, 10% *trans*-dienes and trienes, was found in an oil from a preserve of tuna from Asia. It contained 0.08% of *trans*-18:1 only, but 4.6% of 18:2 isomers (9% of the linoleic acid being isomerized) and 5.4% of *trans*-18:3 (merely 2.9% left in all *cis*).

Proportion of isomerized acids

When fatty acids with at least one *trans* double bond are depicted as percentage of the acid of origin (i.e. the *trans*-dienes as percentage of the sum of the linoleic acid plus its *trans* isomers), results no longer depend on the fatty acid composition of the oil. As shown in figure 4, in more than half of the oils, less than 1% of the linoleic acid was isomerized, independently of whether looking at rapeseed and

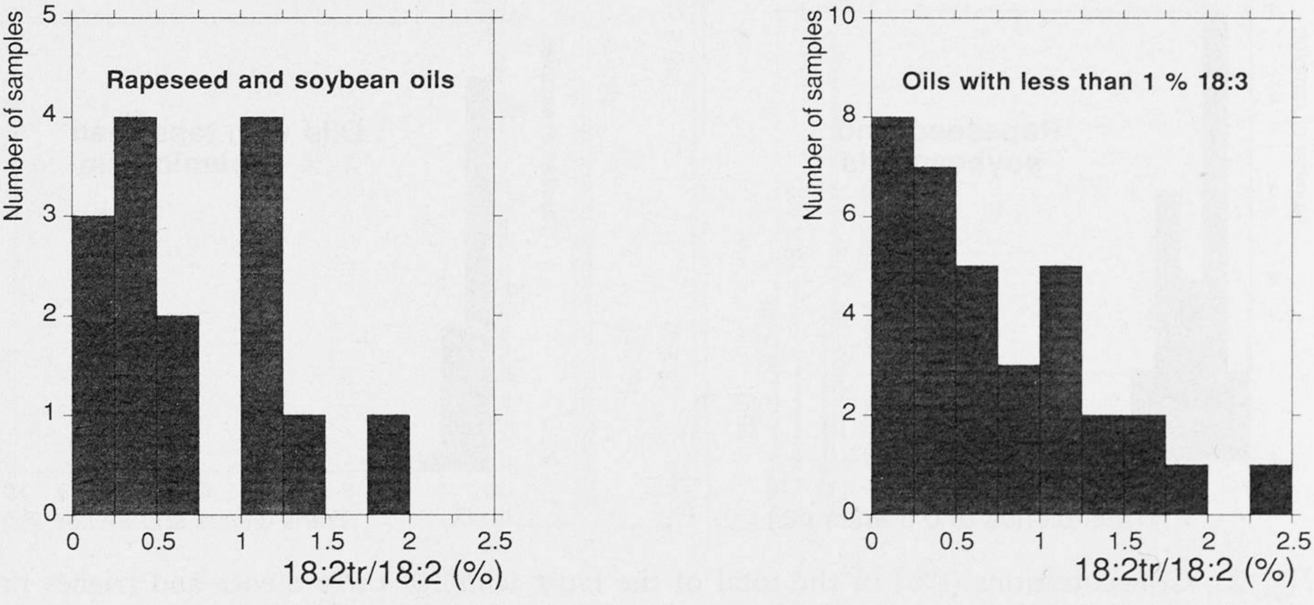

Fig. 4. Percentage of isomerized linoleic acid in rapeseed and soybean oil (left) as well as in all other oils (right)

soybean or at the other oils. Among the 47 samples of bottled oils analyzed, 2.3% was the highest proportion of isomerized linoleic acid.

Figure 5 shows the concentration of *trans*-trienes as percentage of the original linolenic acid for the rapeseed and soybean oils sold in bottles (i.e. disregarding the high values of some oils from the preserves). In more than half of the samples, less than 10% of the linoleic acid was isomerized. There were, however, also two samples with more than 20% *trans* isomerization.

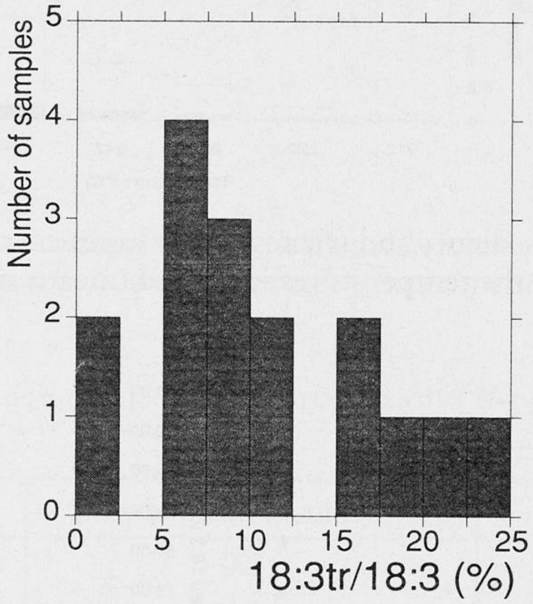

Fig. 5. Percentage of isomerized linolenic acid in rapeseed and soybean oil

Laboratory experiments

Concentration of trans-dienes and trienes

Preliminary experiments confirmed previous reports that isomerization depends on the temperature and the duration of heating applied, but not on passage of gas or vapor. Hence, experiments could be simplified by heating evacuated oil without flow of a stripping medium.

Raw rapeseed oil initially containing 20.2% linoleic acid and 9.4% linolenic acid was heated during 45 min to the temperatures indicated in figure 6. In two independent experiments, heating to 232 °C resulted in 0.52 and 0.65% of *trans*-dienes and trienes. At 142 °C, 0.89% of *trans*-dienes and trienes were formed and 1.5% at 257 °C. Bleached rapeseed oil gave almost identical results.

Proportion of acids in trans

Proportions of isomerized linoleic and linolenic acid shown below refer to experiments performed with raw and bleached rapeseed and soybean oil. As there were no significant differences between them, the data was combined in the same diagrams. Results shown in figure 7 were obtained with treatments lasting 45 min. At 212 °C, isomerization of linoleic acid remained below 0.1%. It reached 1% at

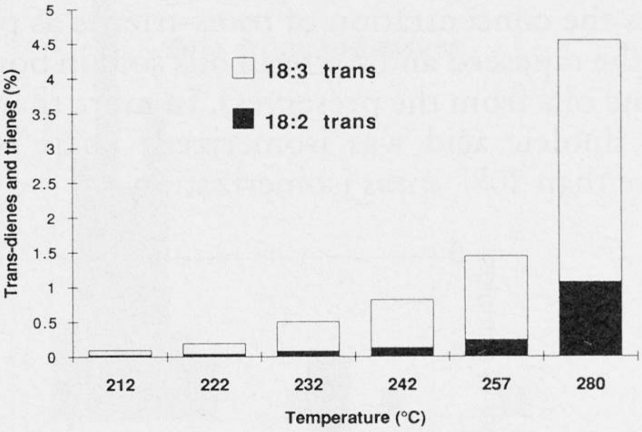

Fig. 6. Formation of *trans*-dienes and trienes in raw rapeseed oil during experimental 45 min heat treatments at the temperatures indicated (means of two determinations)

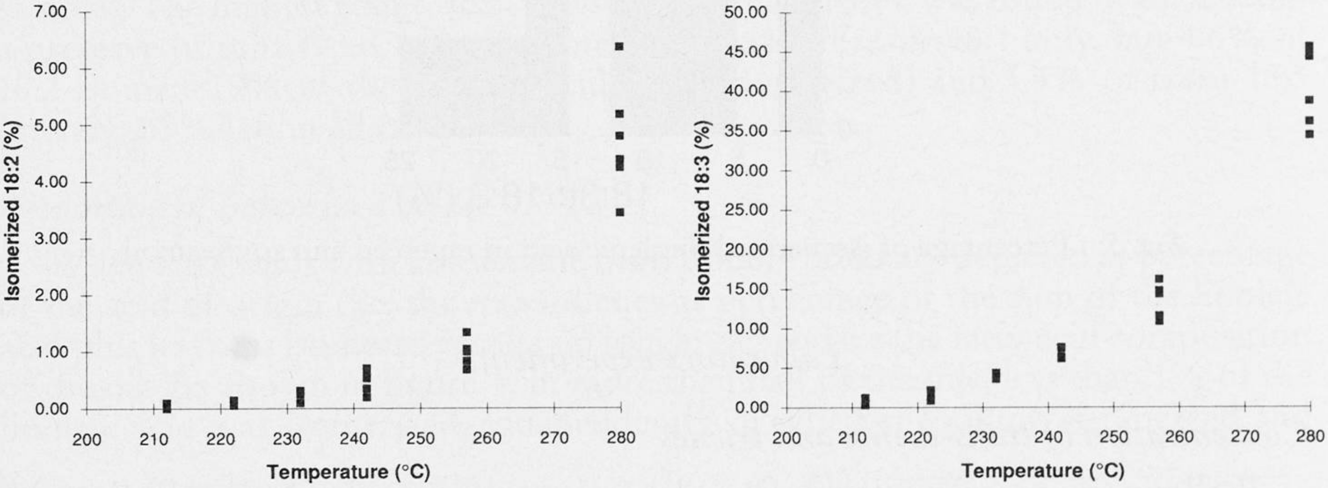

Fig. 7. Proportion of isomerized linoleic acid (left) and linolenic acid (right) after heating 45 min to the temperature indicated (laboratory experiments)

257 °C (ranging from 0.7 to 1.3%) and about 5% at 280 °C. Isomerization of linolenic acid was close to 1% after heating to 212 °C, reached 5% in a temperature range of 235–240 °C, and of 10% at 250–255 °C.

As shown previously (2), cis/*trans* isomerization proceeds at fairly constant rates, i.e. the proportion of isomerized acid increases linearly with time. Figure 8 confirms this for a temperature of 243 °C, though with considerable scattering of the results.

Cis/*trans* conversion modestly depended on the presence of oxygen. A soybean oil was heated at 250 °C during 60 min in a glass tube (i) with half of the tube filled with air, (ii) the same oil being evacuated shortly (5 min, water aspirator), and (iii) rinsed with nitrogen and sealed after longer evacuation. Results shown in table 1

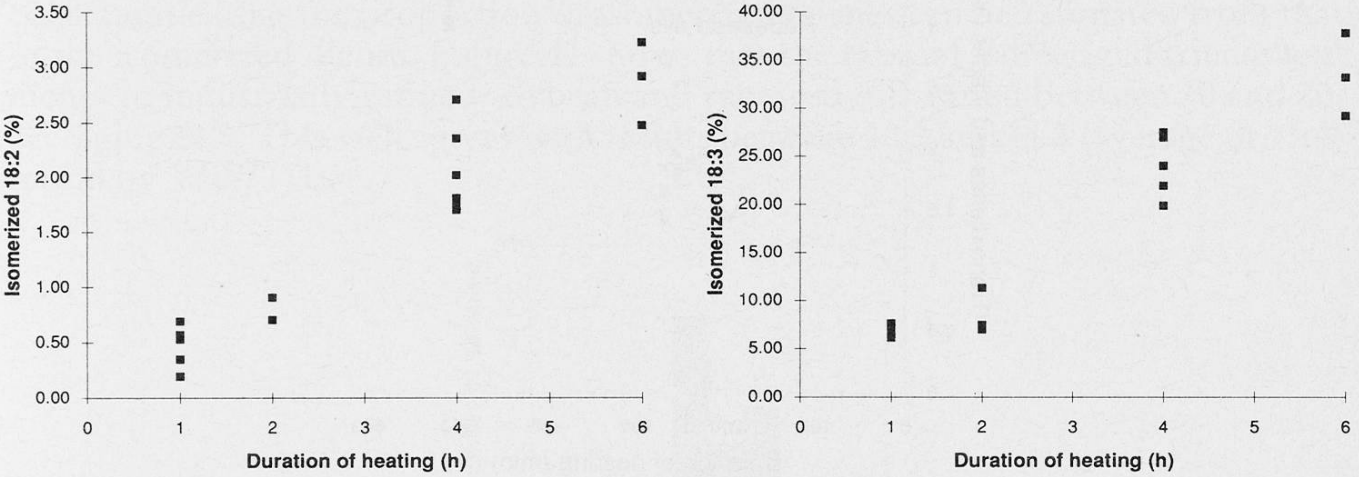

Fig. 8. Proportion of isomerized linoleic acid (left) and linolenic acid (right) after heating to 243 °C for the duration indicated (laboratory experiments)

Table 1. Influence of presence of air on the proportion (%) of isomerized linoleic and linolenic acid

Experiment	Isomerized 18:2	Isomerized 18:3
With air	0.71	8.8
Short evacuation	0.52	6.7
N2, long evacuation	0.55	7.0

indicate some improvement by evacuation, but no further reduction as the removal was performed more carefully.

These results agree with the finding of *Wolff* (2) that *cis/trans* isomerization depends little on the oil and its pretreatment. It can, in fact, be estimated from the temperature and duration of deodorization.

Data from industrial deodorization

Concentration of trans-dienes and trienes

Soybean and rapeseed oils from routine industrial deodorization were analyzed in the same way. Temperatures were around 235 °C, whereas the duration varied between 225 and 270 min. Some oils were deodorized a second time after storage over several years. Durations of the two treatments were added up. As shown in figure 9, the oils contained between 1 and 2% *trans*-dienes and trienes if deodorized once (one exception) and around 3% when deodorized twice, showing that the stress measured in *trans* concentrations adds up. There was little difference between soybean and rapeseed oil: the lower concentration of linolenic acid in soybean oil was compensated by a substantially higher one for linoleic acid.

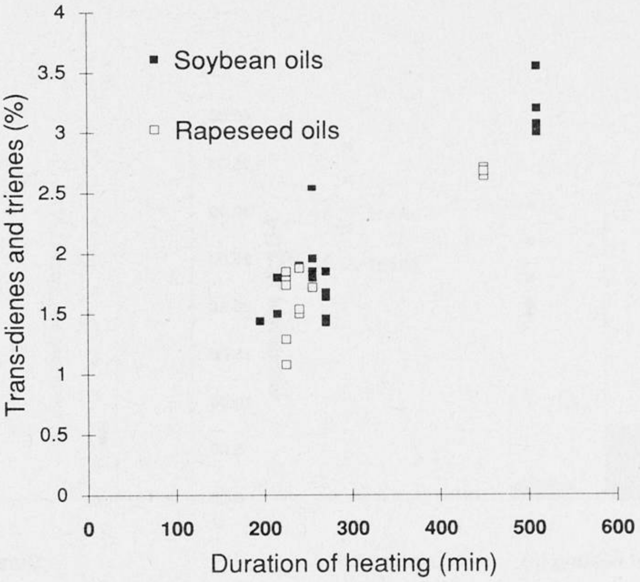

Fig. 9. Sum of *trans*-dienes and trienes in industrially refined soybean and rapeseed oils, involving deodorization at 235 °C for the durations indicated

Proportion of isomerized dienes and trienes

Figure 10 reports the data as percentage of isomerized linoleic or linolenic acid. For linoleic acid in oils refined once, isomerization was around 1% and remained below 1.25% with one exception. Corresponding values for linolenic acid varied between 10 and 20%. Maximum values for doubly refined oils were 2.3 and 36%, respectively.

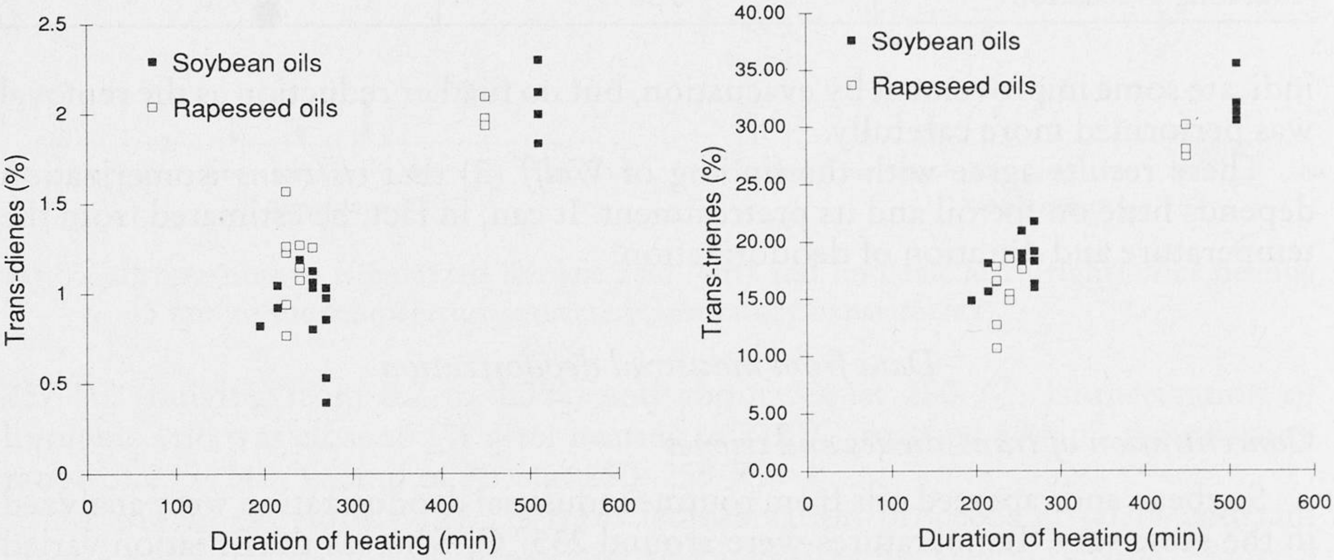

Fig. 10. Percentage of isomerized linoleic acid (left) and linolenic acid (right) in soybean and rapeseed oils refined industrially with deodorization at 235 °C

Ratio of trans-dienes and trienes

Trans-trienes are formed at higher rates than dienes. Kinetics were investigated by *Wolff* (11). As will be shown in the Discussion, it could be useful for routine analysis that the ratio of the isomerization rates of linoleic and linolenic acid is fairly constant, i.e. that the proportion of isomerized trienes can be estimated from that of the isomerized dienes. Figure 11 shows that the ratio of isomerized trienes and dienes in industrially refined soybean and rapeseed oils varied between 10 and 20, averaging 13.9. This well agrees with results between 10.2 and 14.3 (average of 13.4) found by *Wolff* (11).

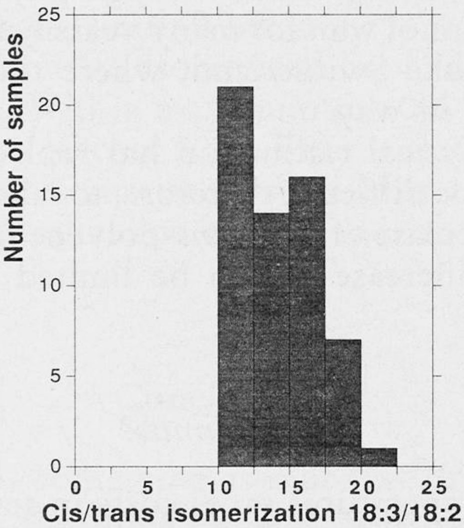

Fig. 11. Ratio of the proportions of isomerized trienes and dienes in industrially refined soybean and rapeseed oils

Discussion

Food control authorities are confronted with the question whether and to what extent the recent strong increase in cis/*trans* isomerization introduced by new raffination procedures should be tolerated. Advantages of physical raffination for industry must be weighed against possible (and unknown) health effects on consumers. For the consumer (to be protected by the food control authorities) it means comparing a (small) financial advantage with a health risk.

A strict defender of the consumer's interests would point to the generally increasing risks by the rapidly evolving food technology and insist in minimizing exposure to new compounds with unknown effects, particularly if consumed in amounts approaching grams. He would ask industry to prove that *trans* isomers of essential fatty acids have no negative health effects before temperature of deodorization is increased as drastically as seems to be necessary to achieve physical raffination (19). Since no concluding toxicological study has been carried out, he would ask industry to return to deodorization at temperatures below 200 °C and to introduce physical refining only after development of a technology avoiding substantially increased *cis/trans* isomerization. If industry insisted in high-temperature-deodorization, a thorough toxicological study on the effects of the *trans*-dienes and trienes would be needed in order to enable the deduction of maximum allowable concentrations.

After decades of intense work, there is still no agreement on the health effects of elaidic acid (just one of the *trans* isomers of oleic acid) (20). Since the *trans*-dienes and trienes consist of six compounds at least, all behaving differently, a toxicological investigation of the many possible effects of *trans*-polyenes is likely to be an extremely expensive and time-consuming task. If the oil industry would be asked to finance such studies, it would probably refuse, because the investment would hardly be returned by the savings made through physical refining. Furthermore, food control authorities cannot wait for many years before making some decisions, particularly in countries like Switzerland, where the change from chemical to physical refining is just on its way.

In many countries, physical raffination has replaced chemical neutralisation almost completely. It will be difficult, therefore, to take the strict position outlined above and a substantial increase of the *trans*-polyene fatty acids must probably be accepted. However, this increase has to be limited to the minimum which is technologically achievable.

Legal limits?

Application of high temperatures saves costs in several ways, e.g. because the process needs less careful control when more than the absolute minimum is applied (i.e. some «safety margin» is included), or physical raffination can be performed in installations which have not been optimally designed for this process. Legal limits are the classical tool to enforce improvement and to avoid that cheaper, but poor products win on the market.

Limits could be imposed in the two ways mentioned above, i.e. as a maximum concentration of the sum of the *trans*-dienes and trienes or as a maximum proportion of isomerized linoleic or linolenic acid. A limited concentration of the summed *trans*-dienes and trienes restricts the total amounts human metabolism has to cope with. Disregarding double raffination of stored oils, the above results indicate that a limit of 2% for rapeseed and soybean oils and of 1% for the other oils would accept most of today's bottled oils of the Swiss market, but only some 65% of the oils in the preserves. A limit of 1% for oils rich in linolenic acid and of 0.5% for the others would reject almost half of the bottled oils. Lower limits could apply for infant foods, considering the importance of the essential fatty acids for growth at that age, e.g. regarding the development of the brain.

A limitation based on the degree of isomerization might, however, be more appropriate. Reasoning might involve the following speculations. As human metabolism needs polyunsaturated fatty acids, it picks them up from the material supplied, while the excess is consumed for energy production. This means that the amount of *trans*-dienes and trienes entering metabolism is largely independent of absolute amounts consumed, but just a function of the degree of isomerization. It may even be argued that a «hungry» metabolism is less selective and incorporates more *trans*-acids. Even when disregarding the latter, this view advocates limits for the proportion of isomerized fatty acids.

As it is analytically difficult to determine the *trans*-trienes in oils containing little linolenic acid, it turns out useful that the isomerization of linolenic acid can be estimated from that of linoleic acid, applying a factor of about 14 (figure 11). Hence it is sufficient to determine the two isomers of *trans*-dienes and to define a legal limit for them.

If 20% isomerized linolenic acid would be considered as limit, also some 1.4% of linoleic acid would be isomerized. Most of the bottled oils presently found on the Swiss market would pass this limit. When only 14% isomerization of linolenic acid is tolerated, recognized by 1% isomerized linoleic acid, about a third of the products would be rejected. With a maximum of 10% isomerized linolenic acid (0.7% *trans*-dienes), rejection reaches 50%. Even with this lowest limit, however, we would accept an increase of isomerized essential fatty acids by a factor exceeding 10 compared to chemical neutralization with deodorization at lower temperature.

Conclusion

The authors consider oils containing as much as 10% of isomerized essential fatty acids (nearly 70% of the linolenic acid with double bonds in *trans*) as unacceptable, nor do they recognize sufficiently important reasons compelling us to accept the strong increase in *trans*-polyene fatty acids.

The high concentrations of *trans*-polyene fatty acids were introduced through the ecologically and economically advantageous physical raffination. If a substantial increase in their concentration must be tolerated owing to the fact that today most of the oils are worked by this procedure, limits should at least require careful optimization of the installations and procedures.

A limit of 1.25% isomerized linoleic acid should be imposed immediately, and a limit of 0.5% foreseen in a few years unless toxicology demonstrates innocuousness of these acids. Infant foods should immediately be required to contain less than 0.5% isomerized linoleic acid (i.e. to contain oil from chemical raffination at least for the moment).

As the formation of *trans*-dienes and trienes is primarily a function of temperature and time, maximum tolerable conditions can be estimated. When 1.25% isomerization of linoleic acid is tolerated, oils may be refined during some 5 h at 235 °C or 1 h at about 255 °C. 0.5% isomerization results from heating during about 2 h at 230 °C or 1 h at 240 °C.

Acknowledgement

Industrially refined soybean and rapeseed oils were from Florin AG, Muttenz, Switzerland.

Summary

During the last years, concentrations of isomerized essential fatty acids (*trans*-dienes and trienes) in edible oils increased by a factor of 10–100 upon changing from chemical to physical raffination (stripping free fatty acids during deodorization). The highest concentration found in a survey of the Swiss market was 10% for the sum of the *trans*-dienes and trienes, with nearly 70% of the linolenic acid being isomerized to acids containing one *trans* double bond at least. Legal limits are suggested forcing refineries to improve on this.

Zusammenfassung

In den letzten Jahren stiegen die Konzentrationen isomerisierter essentieller Fettsäuren (*trans*-Diene und -Triene) in Speiseölen um einen Faktor 10–100 als Folge der Umstellung von chemischer zu physikalischer Raffination (Entfernung freier Fettsäuren über die Desodorierung). Bei einer Übersicht des schweizerischen Markts lag die höchste Konzentration für die Summe aller *trans*-Diene und -Triene bei 10%, wobei annähernd 70% der Linolensäure zu Säuren mit mindestens einer *trans*-Doppelbindung isomerisiert waren. Gesetzliche Limiten werden vorgeschlagen, um die Raffinerien zu einer Verbesserung dieser Situation zu veranlassen.

Résumé

Pendant les dernières années, la concentration des acides gras essentiels isomerisés (*trans*-diènes et -triènes) dans les huiles a augmenté d'un facteur 10–100 en raison du remplacement de la neutralisation chimique par un traitement physique (élimination des acides libres par la déodoration). Lors d'un contrôle au niveau du marché Suisse, la concentration maximum de la somme des *trans*-diènes et des -triènes était de 10%, toutefois environ 70% de l'acide linolénique ont subi une isomérisation en acides avec une double liaison *trans* au moins. Des limites légales sont proposées pour obliger les raffineries d'améliorer la situation.

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