
1. INTRODUCTION, OBJECTIVES OF THE STUDY / BEVEZETÉS, CÉLKITŰZÉSEK

Double bonds of natural unsaturated fatty acids in vegetable oils and fats are in *Z* configuration. Ackman et al. were the first in 1974, appointing that during the refining process under the generally applied deodorization conditions, geometrical isomerization of linoleic and linolenic acid takes place. It was observed that the temperature and the duration of the operation predominantly determine the degree of geometrical isomerization. The quality of refined oils, however, was mainly defined by the organoleptic parameters and the ageing properties.

Because of possible nutritional and health aspects, minor components such as (*E*)-isomer fatty acids, tocopherols, oxidized and polymerized triacylglycerols are more concerned today. During the 1990s in several nutritional studies, negative health effect of (*E*) fatty acids was suggested. Dietary (*E*)-monounsaturated fatty acid isomers formed during partial hydrogenation of vegetable oils have especially been linked with an increased risk of coronary heart disease. Analysis of the daily intake data showed that among the dietary sources of (*E*)-isomer fatty acids partially hydrogenated fats are the most important. These facts led to a trend to decrease the total (*E*)-isomer intake with special regards to the partially hydrogenated fats.

Fewer studies were performed concerning the possible effect of non-hydrogenated polyunsaturated vegetable oils. The main reason to this is that under regular deodorization conditions the (*E*)-isomers of the individual polyunsaturated fatty acids can not be selectively produced in appropriate quantities. In industrial deodorization geometrical isomerization of monounsaturated fatty acids is negligible. Polyunsaturated fatty acids are much more prone to the reaction. Surveying the (*E*)-isomer fatty acid content of refined vegetable oils available in Western-European countries, Wolff (1992 and 1993) found 0.2-1.0% (*E*)-linoleic acid and up to 3% (*E*)-linolenic acid in the products. Studies related to geometrical

isomerization of linolenic acid demonstrated that the reaction follows first order kinetics. The probability of linolenic acid isomerization is 12-14 times higher than that of linoleic acid.

The increasing concerns about the nutritional value of edible oils and the suspiciousness around (*E*) fatty acids resulted in an additional objective to the refining process. For vegetable oil refiners this means an optimization of the process to minimize the formation of (*E*) fatty acids and keep the tocopherol loss as low as possible. Nevertheless, the primary aim of refining remained the production of edible oils with low FFA content and strictly neutral taste and odour. Consequently, a good compromise is necessary to find optimal process conditions.

Detailed kinetic model comparing geometrical isomerization of linoleic and linolenic acid has not been published yet.

The main goal of present study was to develop such model and to apply in the following industrial and research areas:

- Prediction of (*E*)-isomer fatty acid formation under the circumstances of industrial deodorization, and to keep the (*E*)-isomer content of refined vegetable oils as low as possible.
- Characterisation of industrial deodorizers, detection of excessive degree of geometrical isomerization caused by local overheating or inhomogeneous residence time.
- Calculation of deodorization conditions for selective isomerization of linolenic acid, pilot plant scale production of refined oil with special (*E*)-isomer content and composition for an international nutritional study (“Nutritional and Health Impact of *trans* Polyunsaturated Fatty Acids in European Populations”).

Low erucic rapeseed oil was deodorized at laboratory scale to establish the model. The possible degradation of linoleic and linolenic acid was also characterized; its influence on the formation of geometrical fatty acid isomers was taken into account.

The results were validated by batch deodorization of low erucic rapeseed oil at pilot plant scale.

Concerning the (*E*)-isomer content of commercial refined oils in Hungary, no summarizing data are available. Therefore, an expansive survey was made to characterize the (*E*) fatty acid content of imported and locally produced vegetable oils marketed in Hungary. In order to compare the Hungarian data with the current European and American tendency, a similar survey was completed on refined oils from some selected foreign countries.

* * *

A természetben előforduló telítetlen zsírsavak *cisz* konfigurációjúak. Ackman és munkatársai (1974) mutattak rá először, hogy növényolajok finomítása során, az általánosan alkalmazott dezodorálási körülmények között a linol- és linolénsav kismértékű geometriai izomerizációja is végbemegy. Megfigyeléseik szerint az izomerizáció mértékét elsősorban a dezodorálás hőmérséklete és ideje határozza meg. Mindazonáltal a finomított olajok minőségét döntően az érzékszervi tulajdonságok és a tárolhatóság alapján ítélték meg.

A későbbiekben - lehetséges táplálkozástani és egészségügyi hatásuk miatt – bizonyos minor komponensek (tokoferolok, *transz* izomer zsírsavak, oxidált és polimerizált trigliceridek a figyelem középpontjába kerültek. Az 1990-es években több táplálkozástani tanulmány is az *transz* konfigurációjú zsírsavak negatív egészségtani hatását mutatta. Főképp a növényolajok parciális hidrogénezésekor keletkező *transz* monotelítetlen zsírsavak bevitele és szív-koszorúér betegségek kockázata között véltek összefüggést felfedezni. Emellett a napi beviteli adatok elemzése azt mutatta, hogy részlegesen hidrogénezett zsírok a táplálékból származó

transz izomer zsírsavak legjelentősebb forrásai. Ezek a tények a *transz* konfigurációjú zsírsavak (különösen a parciálisan hidrogénezett zsírokból származóak) fogyasztásának csökkenéséhez vezettek.

Kevesebb tanulmány született a nem hidrogénezett növényolajokban található többszörösen telítetlen zsírsavakat lehetséges hatására vonatkozóan. Ennek elsődleges oka az, hogy a szokásos dezodorálási körülmények között az egyes politelítetlen zsírsavak *transz* izomerei nem állíthatók elő szelektíven kellő mennyiségben. Növényolajok ipari dezodorálása folyamán *transz* monotelítetlen zsírsavizomerek nem képződnek számottevő mértékben. A többszörösen telítetlen zsírsavak sokkal inkább hajlamosak geometriai izomerizációra. Wolff 1992 és 1993-ban végzett felmérései alapján a Nyugat-Európai országokban forgalmazott étolajok *transz*-linolsav tartalma 0.2-1.0% között alakult, *transz*-linolénsav tartalma a 3%-ot is elérte. A linolénsav geometriai izomerizációjával kapcsolatos publikációkból kitűnik, hogy a reakció első rendű kinetikát követ. A linolénsav izomerizációjának valószínűsége 12-14-szerese a linolsavénak.

Az étolajok táplálkozási értékével és a *transz* izomer zsírsavak hatásával kapcsolatos kérdések előtérbe kerülésével a finomítás céljai az *transz* izomerek mennyiségének minimalizálásával és a tokoferol veszteség csökkentésével egészültek ki. Mindemellett a finomítás elsődleges célja alacsony szabad zsírsavtartalmú, semleges ízű, szagtalan olaj előállítása maradt. Következésképpen az optimális technológiai paraméterek megtalálásához ésszerű kompromisszumot szükséges kötni.

A linolsav és linolénsav geometriai izomerizációját összehasonlító kinetikai modelt még nem jelentettek meg. Jelen értekezés célja egy ilyen model megalkotása és alkalmazása a következő növényolaj-ipari és kutatási területeken:

- *Transz* izomer zsírsavak mennyiségének előrejelzése az ipari dezodorálási körülmények között, a geometriai izomerizáció mértékének minimalizálása.

- Ipari dezodoráló berendezések jellemzése, helyi túlmelegedés és inhomogén tartózkodási idő által okozott, a vártnál nagyobb mértékű izomerizáció detektálása.
- Dezodorálási körülmények számítása linolénsav szelektív izomerizálására, különleges *transz* izomer tartalmú és összetételű étolaj félüzemi méretű előállítására egy nemzetközi táplálkozástani tanulmány vizsgálataihoz (“Nutritional and Health Impact of *trans* Polyunsaturated Fatty Acids in European Populations” – Politelítetlen zsírsavak táplálkozástani és egészségügyi hatása európai populációkban.).

A modelt kis erukasav tartalmú repceolajjal végzett laboratóriumi dezodorálási kísérletek alapján alkottuk meg. Jellemeztük az adott körülmények között a linol- és linolénsav lehetséges degradációját, illetve annak a geometriai zsírsavizomerek képződésére gyakorolt hatását. A model megbízhatóságát kis erukasav tartalmú repceolajjal végzett félüzemi dezodorálási kísérletekkel ellenőriztük

A magyar kereskedelmi forgalomban kapható finomított növényolajok *transz* zsírsav tartalmára vonatkozóan még nem jelentettek meg részletes adatokat. E hiány pótlására átfogóan vizsgáltuk a Magyarországon forgalmazott import és hazai gyártású étolajokat a geometriai izomerizáció szempontjából. Azzal a céllal, hogy a hazai adatokat összevegyük a világ más országaiban tapasztalható tendenciával, hasonló felmérést végeztünk Európából és az USA-ból származó termékekkel is.

2. LITERATURE OVERVIEW

2.1. MAIN CONSTITUENTS OF LIPIDS

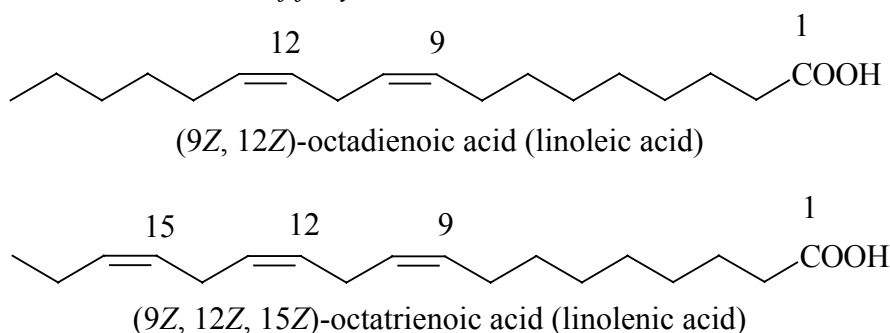
Lipids are often defined as esters of glycerol and fatty acids. The most widely accepted definition identifies lipids according to their solubility as the large group of compounds soluble in hexane but insoluble in water.

Triacylglycerols, triesters of glycerol and long chain aliphatic acids (fatty acids) are the main constituents of lipids. They are always accompanied by numerous minor components such as phosphatides, waxes and the divers compounds of the unsaponifiable fraction.

2.1.1. Fatty acids

Fatty acids are the principal constituents of several lipid classes, they account for 90 to 96% of the molar mass of triacylglycerols. The main fatty acids are generally denoted by their common names or by the shorthand designation, in which the number of carbon atoms and the number and position of the double bonds are given. In systematic nomenclature, the carboxyl carbon is carbon 1 and it defines the main carbon chain:

Figure 1
Structure and nomenclature of fatty acids



According to the so-called ω -numbering, the counting is started from the terminal methyl group of the molecule, which is often more convenient to use.

A large number of saturated and unsaturated fatty acids occur in vegetable oils and fats, their distribution is characterized by the fatty acid composition. These fatty acids have even number of carbon atoms (mostly between 12 and 24) on the chain. Fatty acids also with odd number of carbon atoms can be found in animal fats. The double bonds of unsaturated fatty acids are naturally in *Z* configuration in a methylene-interrupted structure. In Table 1 the fatty acid composition of the most important vegetable oils is summarized.

Table 1
Fatty acid composition^a of the major vegetable oils (Firestone 1999)

Fatty acid	Soybean oil	Palm oil	Rapeseed oil ^b	Sunflower oil
12:0	0-0.1	0-0.4	-	0-0.1
14:0	0-0.2	0.5-2.0	0-0.2	0-0.2
16:0	9.7-13.3	40.0-48.0	3.3-6.0	5.0-8.0
16:1	0-0.2	0-0.6	0.1-0.6	0-0.3
17:0	-	-	0.3	-
18:0	3.0-5.4	3.5-6.5	1.1-2.5	2.5-7.0
18:1	17.7-28.5	36.0-44.0	52.0-67.0	13.0-40.0
18:2	49.8-57.7	6.5-12.0	16.0-25.0	48.0-74.0
18:3	5.5-9.5	0-0.5	6.0-14.0	0-0.3
20:0	0.1-0.6	0-1.0	0.2-0.8	0.2-0.5
20:1	0-0.3	0-0.2	0.1-3.4	0-0.5
20:2	0-0.1	-	0-0.1	-
22:0	0.3-0.7	0-0.1	0-0.5	0.5-1.3
22:1	0-0.3	-	0-4.7	0-0.5
24:0	0-0.4	0-0.2	0-0.2	0-0.4

^a % related to the total amount of fatty acids

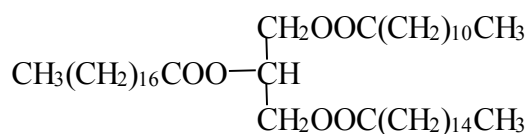
^b Low erucic rapeseed oil

2.1.2. Acylglycerols

Approximately 98% of fats and oils are composed by triacylglycerols or

triglycerides, where the glycerol molecule is linked to three fatty acids. The Fischer planar representation, in which the secondary alcohol group is positioned on the left side of the carbon chain, provides the most common manner of denoting triacylglycerols. The carbon atoms are numbered 1, 2 and 3 in the upper, middle, lower carbon order, thus the compound in Figure 2 is named glycerol-1-stearate-2-palmitate-3-oleate.

Figure 2
Structure of triacylglycerols



The large variety of fatty acids and the number of possible combination with glycerol molecule make fats and oils a complex mixture of triacylglycerols. The way in which fatty acids combine with glycerol as well as the type and proportion of fatty acids have a large influence on the physical and chemical properties of fats and oils.

Acylation of glycerol with only one or two fatty acids yields partial acylglycerols that can also be found in fats and oils. The presence of mono- and diacylglycerols is a marker of a not completed triacylglycerol synthesis in oleaginous seeds and fruits or can be a consequence of hydrolytic reactions provoked by enzymes.

2.1.3. Minor components

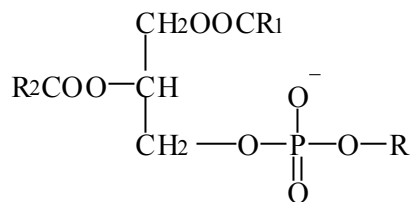
Beside triacylglycerols vegetable oils and fats contains a great number of compounds in different, sometimes even in significant quantities. A brief overview of natural minor components is given here focusing on phospholipids, waxes and

the main components of the unsaponifiable matter such as sterols, tocopherols, hydrocarbons, triterpenic alcohols and pigments.

2.1.3.1. Phospholipids

Phospholipids are a group of compounds derived from phosphoryl-3-glycerol. Acylation of phosphoryl-3-glycerol by two fatty acid molecules forms phosphatidic acids. Most of the phospholipids present in fats and oils are phosphatidyl esters, derivatives of phosphatidic acid, in which the phosphate group is also esterified with a hydroxy compound. As it is shown in Figure 3, these compounds can be either amino alcohols (choline, ethanolamine, serine) or polyalcohols (inositol).

Figure 3
Phospholipids in vegetable oils



R	Name
H	Phosphatidic acid
CH ₂ CH ₂ -NH ₃ ⁺	Phosphatidylethanolamine
CH ₂ CH ₂ -N ⁺ (CH ₃) ₃	Phosphatidylcholine
$\begin{array}{c} \text{CH}_2\text{CH}-\text{NH}_3^+ \\ \\ \text{COO}^- \end{array}$	Phosphatidylserine
C ₆ H ₁₁ O ₅	Phosphatidylinositol

2.1.3.2. Waxes

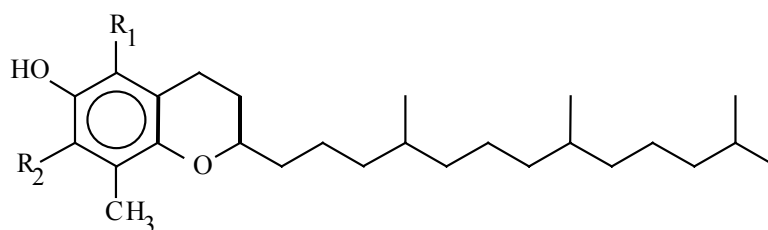
Natural waxes are esters of long chain fatty acids (20-28 carbon atoms) and long chain aliphatic monohydric alcohols (22-30 carbon atoms). They can be found both vegetable oils (sunflower, olive or cottonseed oil) and in animal fats (higher marine animals and fish). Waxes have an important role in vegetables forming protective

coating of fruits and seeds.

2.1.3.5. Tocopherols

Vegetable oils are important sources of natural tocopherols. The α -, β -, γ - and δ -tocopherols are specified according to the number and location of methyl groups on the chromanol group in the positions 5 and 7 as shown in Figure 4. The polyisoprenic side chain can be either saturated (tocopherols) or triunsaturated (tocotrienols).

Figure 4
Chemical structure of tocopherols



	R ₁	R ₂
alpha	-CH ₃	-CH ₃
beta	-CH ₃	-H
gamma	-H	-CH ₃
delta	-H	-H

Tocopherols are biologically active substances, they show vitamin E activity. The International Unit (IU) of the activity is defined as the activity of 1 mg d,l- α -tocopheryl acetate. The vitamin E activity of tocopherol homologues decreases in the α to δ order. These compounds are also very important natural antioxidants. They are called radical scavengers as they act by giving one or two hydrogen atoms per molecule to free radicals present in the medium, stopping one or two radical reactions in this way. Their *in vitro* antioxidant efficiency decreases in the $\delta > \gamma = \beta > \alpha$ order.

The typical tocopherol content of some vegetable oils is given in Table 2.

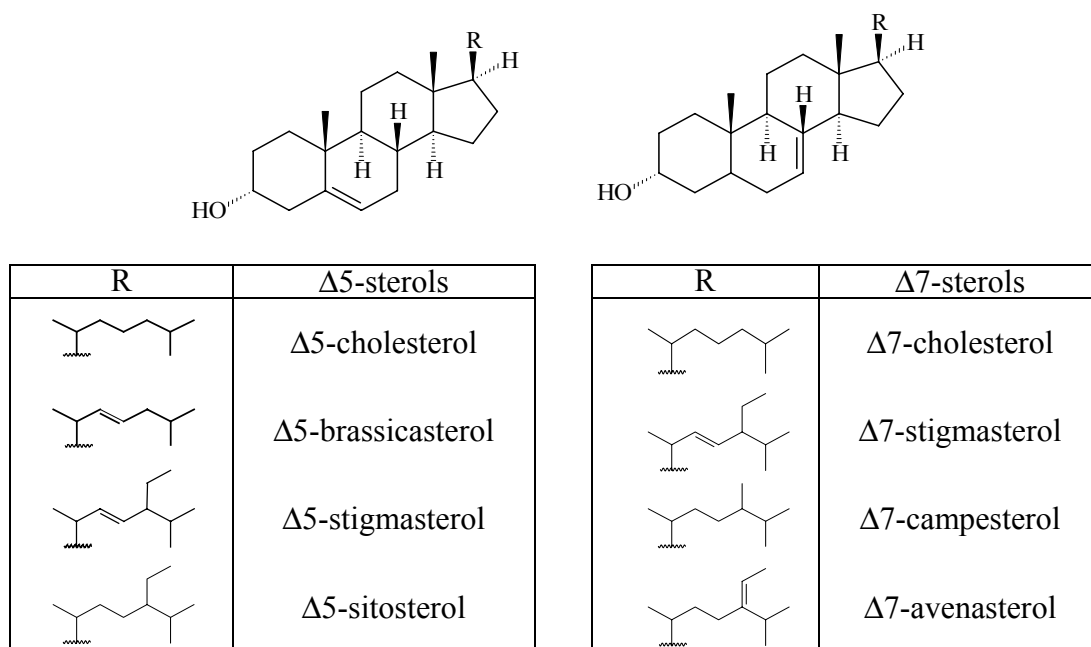
Table 2
Tocopherol and sterol content of crude vegetable oils

	Unsaponifiable material, %	Tocopherols mg/kg	Sterols mg/kg
Soya (Pouzet 1996)	0.5-1.6	800-1600	2500-4180
Palm (Wuidart 1996)	0.5-1.2	320-1000	400-900
Rape (Morice 1996)	0.7-1.8	600-870	5400-8800
Sunflower (Merrien 1996)	0.5-1.5	440-1200	3250-5150

2.1.3.3. Sterols

Sterols are tetracyclic compounds consisting of 27 to 29 carbon atoms (Figure 5). They all possess a hydroxy group at carbon 3 and a branched aliphatic chain at carbon 17. In most cases the tetracyclic ring contains an ethylene bond located more frequently at 5, but can also be found at carbon 7 ($\Delta 5$ and $\Delta 7$ sterols).

Figure 5
The most important sterols of vegetable oils



Sterols account for about 30-60% of the unsaponifiable matter. The dominant sterol compound, characteristic to animal fats is cholesterol, which makes up more than 98% of total sterols. Cholesterol is not specific to animals as believed for a long time, it can also be found in small amounts in numerous plants. Plant sterols are increasingly used in anti-cholesterol diet and in production of pharmaceuticals.

The most abundant phytosterols of vegetable oils are sitosterol, campesterol and stigmasterol of $\Delta 5$ family, $\Delta 7$ -avenasterols and $\Delta 7$ -stigmasterols are present in smaller quantity. The sterol composition is characteristic to a given oil and this fact is often used in detection of adulteration.

2.1.3.4. Triterpenic alcohols

The pentacyclic and tetracyclic triterpenic alcohols originate from squalene after polycyclization. They can be found in higher amount in olive oil and corn oil. Cycloartenol and 24-methylene-cycloartanol, forerunners of sterols occur almost in all vegetable oils.

2.1.3.6. Hydrocarbons

Fats and oils always contain small quantity of hydrocarbons that can be saturated or unsaturated, aliphatic and terpenic origin. The most important compound of this group is squalene, a polyisoprenoid hydrocarbon with thirty carbon atoms. It is present in significant proportion in shark liver oil and olive oil. After hydrogenation to squalane it is widely used in the cosmetic industry.

2.1.3.7. Pigments

The color of fats and oils is highly determined by lipophilic pigments present in trace amounts.

Carotenoids.

The group of carotenoids includes carotenes, which are hydrocarbons, xantophylls

possessing oxygenated groups and different degradation products. Carotenes contain a large number of conjugated double bonds, which contributes to the color of vegetable oils in the yellow-red region of the visible spectrum. The major carotenes of vegetable oils are α -, β - and γ -carotenes, of which the β -isomer has the greatest proportion. Palm oil contains exceptionally high quantity, 500-800 mg/kg carotenes. Being the precursors of vitamin A, α - and β -carotenes are referred to as provitamin A.

Chlorophylls.

The green color of olive, grape and rapeseed oil is attributed to chlorophyll *a* and *b*. Concerning their structure, chlorophylls consist of a porphyrin ring with a magnesium cation in the central position. Loosing the magnesium ion, for example during oil processing, they transform to the brown colored pheophytin *a* and *b*, which are also oil soluble.

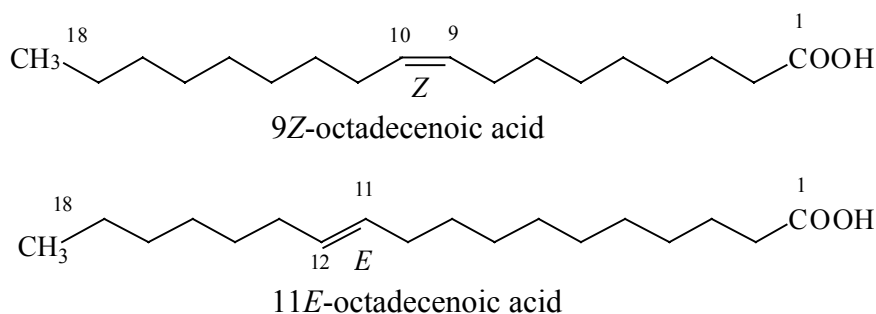
2.2. (E)-ISOMER FATTY ACIDS

2.2.1. General characteristics

Double bonds of natural unsaturated fatty acids are generally in the *Z* configuration, meaning that the higher order substituents are on the same side of the double bond. Figure 6 illustrates the structural difference between the (*Z*) and (*E*) geometrical isomers. Thermodynamically the *E* configuration is more stable, which explains that formation of (*E*)-isomers is favoured during the deodorization and hydrogenation processes and their lower reactivity compared to the corresponding (*Z*)-isomers. The (*E*)-isomers form weaker complexes with metal ions, which is the principle of their separation by argentation thin-layer chromatography. Also, physical properties of (*Z*)- and (*E*)-isomers show differences. The melting point of the geometrical isomers and their saturated equivalent increases in the

$Z < E < \text{saturated}$ order.

Figure. 6
Chemical structure of *Z* and *E* isomer fatty acids



2.2.2. Dietary sources of *E* fatty acids

(*E*)-isomer fatty acids have two primary sources. Firstly, resulted by a biohydrogenation process, meat and diary products from ruminants contain naturally significant amount of monounsaturated fatty acids. Secondly, during processing of fats and oils (deodorization and especially partial hydrogenation) divers (*E*) fatty acids form in different quantities. High quantity, predominantly monounsaturated (*E*) fatty acids are present in partially hydrogenated fats, whereas refined vegetable oils contain (*E*)-polyunsaturated fatty acids in relatively small quantities.

2.2.2.1. Ruminant fats

Ruminant fats are the main source of naturally occurring (*E*)-isomer fatty acids. These compounds result from the biohydrogenation of polyunsaturated fatty acids. As early as in 1928 Bertram isolated vaccenic acid, (*E*11)-18:1 denoted from the Latin *vacca*, cow. (*E*)-18:1 isomers of position 5 to 16 were later identified in goat and cow milk fat and adipose tissues. Beyond the feed, this complex composition of

(*E*)-isomers is influenced by several factors such as the lactation period, breed and the hydrogenation activity of the rumen's microorganisms (Wolff et al., 1998).

Ruminant meat lipids and depot fats

(*E*)-18:1 acids of beef are originated from the lean part of the meat, the fat surrounding the cut and from other fatty tissues used for tallow production (Table 3). (*E*)-octadecenoic acids tend to be present in higher amounts in the adipose tissues. Analyzing six raw beef sample Lin et al. (1984) found a mean of $1.7 \pm 0.9\%$ (*E*)-18:1 acid in the separated lean part and $3.1 \pm 1.4\%$ in the surrounding fat. In frame of an extensive study Slover et al. (1987) analyzed 269 samples of the lean portion of the cuts and obtained an average of 3.2% (*E*)-18:1 acid, but they found approximately double amount in the fatty tissues. Depending on the cooking habits an important proportion of these fats can be ingested contributing to the total (*E*)-octadecenoic acid intake from beef.

Table 3

Dietary sources of (E)-18:1 acids: Ruminant meat lipids and depot fats

Origin	Reference	n ^a	(<i>E</i>)-18:1 isomers, % ^b
Beef meat lipids	Lin et al. (1984)	6	1.7 ± 0.9
Beef fatty tissues	Lin et al. (1984)	6	3.1 ± 1.4
Beef meat lipids	Slover et al. (1987)	269	3.2 ± 1.0
Beef fatty tissues	Slover et al. (1987)	269	6.5 ± 0.3
Beef meat lipids	Leth et al. (1998)	39	2.1 ± 0.9
Veal meat lipids	Leth et al. (1998)	20	4.0 ± 1.2
Lamb meat lipids	Leth et al. (1998)	34	4.5 ± 0.6

^a number of samples

^b as weight percent of total fatty acids (mean \pm standard deviation)

Concerning the distribution of the individual (*E*)-18:1 isomers, practically no difference was noticed for meat lipids and tallow. In both cases vaccenic acid was the predominant isomer representing roughly half of the total *E* isomers.

In a recent study, Leth et al.(1998) compared the fatty acid composition of ruminant

meats analysing 39 samples of beef, 20 samples of veal and 34 samples of lamb. Beef lipids were found to have significantly lower amount of *E* 18:1 acid than veal and lamb (Table 3).

Milk fats

Ruminant milk in all its forms such as milk, butter, cream or cheese is an important dietary source of fats. In France the cow milk fat intake reaches 40-45 g/person/day accounting for one-half of the total daily fat and oil intake. Consequently, an important portion of *E* 18:1 acid intake can be originated from cow milk.

Numerous data have been reported on (*E*) fatty acids in cow milk fat ranging from 2 to 12g per 100g fat (Smith et al. 1978, Deman and Deman 1983). This wide range of the results can partly be explained by the profound influence of feeding conditions and partly it derives from the applied analytical techniques (Henninger and Ulberth 1994). Analysing the amount of (*E*)-18:1 isomers in 1756 German milk samples, Precht and Molquentin (1996) obtained an average value of $3.62 \pm 1.22\%$. The principal isomer was vaccenic acid representing 43.2% of the total (*E*)-octadecenoic acids.

Goat and ewe milk fats are also dietary sources of (*E*)- 18:1 acids. Wolff (1995) has completed a study on French goat and ewe cheeses and reported an average (*E*)-18:1 isomer content of $2.7 \pm 0.9\%$ (n=7) and $4.5 \pm 1.1\%$ (n=8) respectively.

Table 4

Dietary sources of (E)-18:1 acids: Ruminant milk fats (Wolff 1995)

Origin	n ^a	(<i>E</i>)-18:1 isomers, % ^b
Cow milk fat	6	1.7 ± 0.9
Goat milk fat	20	4.0 ± 1.2
Ewe milk fat	34	4.5 ± 0.6

^a number of samples

^b as weight percent of total fatty acids (mean \pm standard deviation)

Comparing these results with those of cow milk fats (Table 4), the (*E*)-octadecenoic acid content of ruminant milk fats decreases in the ewe > cow > goat order.

2.2.2.2. Partially hydrogenated fats

Among the sources of dietary *E* fatty acids partially hydrogenated fats are undoubtedly the most important, usually they contain 25-45% (*E*)-isomers. Beside the addition of hydrogen to ethylene bonds, formation of positional and geometrical isomers is a characteristic feature of nickel catalyzed partial hydrogenation. Allen and Kiess (1955) were the first reporting about formation of geometrical and positional isomers due to a hydrogenation-dehydrogenation mechanism at the surface of nickel catalyst.

Having higher melting point compared to the corresponding *Z* analogues, (*E*) fatty acids used to be popularly applied to improve the physical properties of fat blends. Although concerns about the health aspects of (*E*) fatty acids have increased, nickel catalyst of hydrogenation has remained.

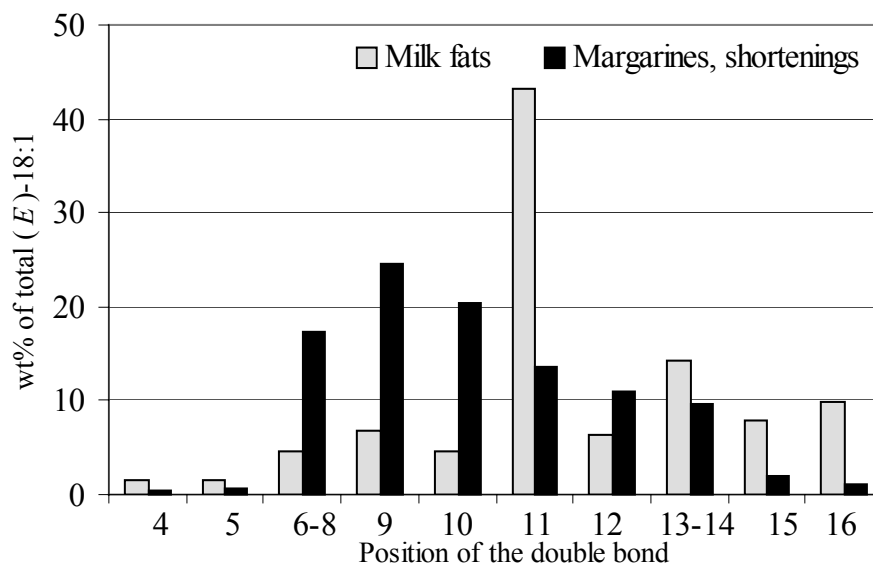
Temperature and pressure are the two parameters of hydrogenation, which are fine-tuned to achieve the desired selectivity and (*E*)-isomer content within the range achievable with nickel catalyst. Applying selective conditions (200-215°C, 100-200 kPa) formation of *E* isomers is promoted compared to non-selective hydrogenation (165-180°C, 300 kPa). According to Bansal and deMan (1982) the use of selective conditions results in 24-33% higher (*E*)-isomer content at similar iodine values during hydrogenation of canola oil.

Wolff et al. (1998) drew attention to the considerable difference between the distribution of the individual (*E*)-18:1 isomers of margarine and milk fats (Figure 7). The difference found between partially hydrogenated oils and ruminant fats did not concern the diversity of individual (*E*)-18:1 acid isomers, but their distribution profile. Contrary to milk fat, the proportion of vaccenic acid in partially hydrogenated fats was only 13.7% (relative to the total (*E*)-18:1 acid content). On the other side the sum of (6*E*)- to (10*E*)-18:1 isomers accounted for 62.2%, while the corresponding value for milk fat was only 15.2%. According to these data it might be possible that the health impact of partially hydrogenated fats are linked to

one or a few specific (*E*)-isomers rather than to these compounds as a whole.

Figure 7

Distribution of (*E*)-octadecenoic acid positional isomers in German milk fats^a and German margarines^b



^a Precht and Molkenin (1996), ^b Precht and Molkenin (1995)

E fatty acid content of margarines may vary in a wide range depending mainly on their types (stick or tube), regional differences in the used fats (vegetable or fish oils) and marketing-economical considerations concerning the assessment of the fat phase. Stick type margarines, usually made of partially hydrogenated fats contain high amount (*E*) fatty acids. A typical range reported by Enig et al. (1983) was 15.9-31.0% (related to the total amount of fatty acids). He also noticed that tube type products, usually mixtures of partially hydrogenated and non-hydrogenated oils have lower (*E*)-isomer content, ranging from 6.8 to 17.6%. Since the 1990s, more attention is paid to *E* fatty acids resulting in a considerable decrease in (*E*) fatty acid level of margarines. Bayard and Wolff (1995) showed a trend towards the so-called “zero-*trans*” margarines: between 1990 and 1994, the (*E*) fatty acid content of French margarines decreased from 3.0-24.0% to 0-17.6% respectively. In

a recent study on the Danish market, Ovesen et al. (1998) found a (*E*)-18:1 acid content of 0-14.2% in hard margarines (n=32) and 0-1.9% in soft types (n=8).

2.2.2.3. Refined non-hydrogenated oils

Ackman et al. (1974) already observed that geometrical isomerization of linoleic and linolenic acid occurs during deodorization. According to Wolff (1992), (*E*)-linoleic acid isomers account for 0.2-1.0% of the total fatty acids, whereas (*E*)-isomers of linolenic acid may add up to 3% in refined vegetable oils. The (*E*) fatty acid content of refined oils mostly depend on the fatty acid composition of the oil and the temperature and time of deodorization. The influence of deodorization conditions on geometrical isomerization will be discussed in chapter 4.1.2.

The popular food frying techniques are considerable sources of dietary (*E*)-polyunsaturated fatty acids. Most of these isomers are already present in the fresh oil if a refined oil is used for frying, but they can also be formed during the frying operations (Chardigny et al. 1996). After 70-hour frying at 170, 180 and 190°C, Tyagi and Vasishtha (1996) found that (*E*)-isomer fatty acid content of soybean oil increased by 1.7, 1.8 and 2.6 % on the total amount of fatty acids respectively.

2.2.3. Daily intake

The (*E*) fatty acid intake can be estimated according to the following approaches: (i) estimates based on “food disappearance”, (ii) analysis of dietary consumption data of a representative population, (iii) laboratory analysis of duplicate portion or composite diets, and (iv) estimates based on the (*E*) fatty acid content of biological tissues (Craig-Schmidt 1998).

Summarizing the studies on the daily intake of (*E*) fatty acids in the industrialized countries, Hayakawa et al. (2000) found large differences. Less than 2 g per capita daily intake was reported in Japan, 5-8 g in the U.S. and up to 13 g in the UK. In the

recent TRANSFAIR study report (Hulshof et al. 1999), daily (*E*) fatty acid consumption in 14 Western European countries were calculated based on the analyses of 100 representative food samples. The values ranged between 1.4 g in the Mediterranean countries and 5.4 g in Iceland, representing 0.5 and 2.1% of the total energy intake respectively. According to Fritsche (1997) the daily (*E*) fatty acid intake estimated from 139 German foods was 1.9 g/person for women and 2.3 g/person for men, which corresponds to a 40% decrease since 1992.

2.2.4. Health impact of (*E*)-isomer fatty acids

2.2.4.1. Metabolism

According to the comprehensive studies by Emken (1991), *E* fatty acids should be considered as a special group of compounds that are recognized, metabolized and regulated by the same mechanism controlling the metabolism of other dietary fatty acids.

Absorption and incorporation

Emken et al. (1989) showed that (*E*)-18:1 fatty acids are well absorbed by humans (90-100%) and incorporated in triglycerides, phospholipides and cholesterol fatty acid esters in a similar way to that of the (*Z*)-isomers. After being absorbed and transferred to the lymph, (*E*)-isomers are transported to different tissues for deposition or catabolism.

Oxidative degradation

Catabolism of (*E*) fatty acids takes place through β -oxidation. The pathway depends on the configuration and the position of the double bond. Human studies showed that (*E*)-18:1 acids are removed from plasma triglycerides more rapidly than oleic acid (Emken et al. 1986 and 1987). A preferential oxidation of (9*Z*,12*E*)-linoleic acid compared to the corresponding (*Z*)-isomer was also demonstrated by S eb edio and Chardigny (2000).

2.2.4.2. Effect of (*E*) fatty acids on polyunsaturated fatty acid metabolism

Mahfouz et al. (1980) characterized the influence of (*E*)-octadecenoic acids on desaturation and elongation. The (3*E*)-, (4*E*)-, (7*E*)- and (15*E*)-18:1 isomers proved to be strong inhibitors of the $\Delta 6$ desaturase enzyme, $\Delta 5$ and $\Delta 9$ desaturases were also inhibited. As a control, no effect of 18:0 was observed on $\Delta 6$ and $\Delta 5$ desaturases and only a slight inhibition of the $\Delta 9$ desaturation was found. On the other hand, inhibitory effect of some *Z* 18:1 isomers (especially the (8*E*)-, (10*E*)- and (11*E*)- isomers) was also noticed. Cook and Emken (1983) also found that at high intake (*E*)-18:1 isomers can inhibit the conversion of linoleic acid to long chain polyunsaturated fatty acids through competition for the $\Delta 5$ - and $\Delta 6$ -desaturases.

E polyunsaturated fatty acids interfere with the metabolism of polyunsaturated fatty acids in a greater extent than (*E*)-monoenes. Their conversion is influenced by the position of the *E* double bond. The (9*Z*,12*E*)-linoleic acid is supposed to be a stronger competitive inhibitor than the (9*E*,12*Z*)-isomer for the conversion of (9*Z*,12*Z*)-18:2 to 20:4. It is 10-20 times more desaturated in position 6 than the 9-*Z*, 12-*Z* isomer (Sébédio and Chardigny 2000).

Concerning *E* isomers of linolenic acid, the (9*Z*,12*Z*,15*E*)-isomer is desaturated and elongated into (17*E*)-isomers of eicosapentanoic and docosahexanoic acid in humans. As Bretillon et al. (1999) demonstrated, polyunsaturated fatty acid isomers possessing *E* double bond in position 9 are dominantly converted into dead end products: (*E*)-20:2 and (*E*)-20:3 isomers.

According to Zevenbergen and Haddeman (1989) (*E*) fatty acids have no effect on eicosanoid biosynthesis when at least 2% of the total energy intake is covered by linoleic acid. In accordance with this, Hayakawa et al. (2000) concluded that as most humans have a relatively high linoleic acid intake, moderate amount of (*E*) fatty acids have no practical influence on the conversion process. Scrimgeour et al. (2001) demonstrated that a diet rich in (*E*)-linolenic acid (0.6% of energy intake)

does not inhibit $\Delta 5$ and $\Delta 6$ desaturation in middle aged man consuming a linoleic acid rich diet.

2.2.4.3. *E* fatty acids and coronary heart disease

The influence of (*E*) fatty acids on serum cholesterol was already investigated in the seventies. This subject received a particular attention because serum cholesterol is considered an important risk factor associated with coronary heart disease (CHD). In early human studies, (*E*) fatty acids had no measurable effect on the total serum cholesterol level (Matson et al. 1975).

Since Mensik and Katan (1990) demonstrated the adverse effect of (*E*) fatty acids, several studies have been performed. Mensik and Katan tested three standardized diets, (i) rich in cholesterol raising saturated fatty acids, (ii) rich in oleic acid, or (iii) in elaidic acid (10% of daily energy intake) in 59 healthy man and woman. Serum LDL- and HDL-cholesterol level were significantly different for the three diets. Compared to oleic acid diet, an (*E*) fatty acid diet increased the LDL-cholesterol and decreased the HDL-cholesterol level compared to oleic acid diet. The same effect on LDL-cholesterol was found in case of saturated fatty acid diet. Consequently, the LDL-/HDL-cholesterol ratio was the highest for the elaidic acid diet. This study was criticized for two main reasons. First, the amount of (*E*) fatty acids in the test exceeded those in common diets. Secondly, contrary to the practice, (*E*)-isomers were obtained by isomerization of high oleic sunflower oil and not by hydrogenation.

Zock et al. (1993) therefore completed a similar study to investigate the effect of (*E*)-18:1 acid at lower intake. In this study, 56 healthy volunteers consumed three different diets in random order. In the first one 7.7% of the daily energy intake was covered by (*E*)-18:1, which was replaced by stearic acid or linoleic acid. The results showed an unfavorable effect of (*E*)-18:1 on serum lipoproteins even at lower intake.

Judd et al. (1994) examined the influence of (*E*)-monounsaturated fatty acids from hydrogenated vegetable oils. Diets with moderate and high (*E*)-isomer intake as well as cholesterol-raising saturated fatty acid diet and oleic acid diet were randomly given to 29 healthy man and 29 healthy woman. In the (*E*)-isomer diets 3.1 and 6.0% of the energy intake was covered by (*E*)-18:1 acids. Compared to the oleic acid diet, both (*E*)-isomer diets raised the LDL cholesterol level while the concentration of HDL cholesterol slightly decreased.

Looking at large numbers of subjects during long period, epidemiological studies provide more reliable information on the relationship between (*E*) fatty acid intake and CHD risks. In the Nurses' Heart Study, Willet et al. (1993) calculated the (*E*) fatty acid intake of 85 095 woman based on food frequency questionnaires. During 8 years of follow up the new cases of CHD enabled them to make a correlation between (*E*) fatty acid intake and CHD. After adjustment for age and total energy intake, a significant positive correlation between the (*E*) fatty acid intake and CHD incidence was found. Additional adjustment for other CHD risk factors, intakes of saturated and monounsaturated fatty acids and linoleic acid did not change the observed correlation. The results of the study also suggest that (*E*) fatty acids from partially hydrogenated fats are more harmful than those from ruminant sources. The above findings were confirmed by Hu et al. (1997) after 14-year follow-up of the same cohort. However, the study can be criticized because there were not enough accurate data on (*E*) fatty acid content of foods available for a reliable, long-term estimation of (*E*) fatty acid intake. According to Stanley (1999) it can only be concluded that an association between the cardiovascular risk and the (*E*)-isomer intake exist, but a cause-effect relationship can not be established.

In frame of the Euramic study, Aro et al. (1995) exhaustively investigated the possible correlation between the risk of CHD and the (*E*) fatty acid level of adipose tissue in eight European countries and Israel. Adipose tissue samples were available from 671 man with a first acute myocardial infarction and 717 controls without

myocardial infarction. No overall difference in the (*E*)-18:1 isomer content of adipose tissue between the myocardial infarction patients and the control group was found. However huge differences in the (*E*) fatty acid level was noticed between the countries involved in the study. The (*E*) fatty acid intake was the lowest in Spain and the highest in Norway and the Netherlands. In Finland and Norway the (*E*)-18:1 acid content of the patients' adipose tissue was significantly higher compared to the control group. This correlation between the adipose tissue *E* fatty acids and CHD risk in Northern Europe led to the presumption that (*E*)-isomers of marine origin might be more atherogenic than those from other sources. There are still not enough data available to support this hypothesis.

2.2.5. Current recommendations concerning *E* isomers

According to the recent FAO/WHO (1995) recommendations with respect to (*E*)-isomer fatty acids:

- food manufacturers should reduce the level of (*E*) fatty acids originated from hydrogenation,
- a governmental monitoring of (*E*)-isomer content in foods is recommended,
- governments should not allow products, which are rich in (*E*) fatty acids, to be labelled as “low in saturates”.

In 1995 the Danish Nutrition Council considered the average (*E*) fatty acid intakes harmful and proposed to reduce the level of (*E*)-isomers in margarine products to less than 5% (Ovesen et al. 1998).

Following the recent reports concluding that (*E*)-isomer fatty acids increase the LDL cholesterol level in the blood and the risk of coronary heart diseases, FDA proposed in 1999 that products containing less than 0.5% (*E*)-isomer fatty acids could be labeled as “*trans*-free” (Hayakawa et al. 2000).

2.2.6. Conjugated linoleic acid

Conjugated linoleic acid (CLA) refers to a group of compounds containing positional and geometrical isomers of octadecadienoic acid with conjugated double bond. Since the 1990's CLA receives particular interest because of their biological effect. Physiological studies showed that CLA is a potent cancer preventive agent in animal models (Ip et al. 1994, Cannella et al. 2000). Their beneficial influence on atherosclerosis (Kritchevsky et al. 2000, Wilson et al. 2000) and on body fat deposition has also been demonstrated (Park et al. 1999, Gavino et al. 2000).

The predominant dietary sources of CLA are ruminant based products. In milk 0.34-1.20%, in meat products 0.1-1.2% of the total fat is represented by these compounds (Fritsche et al. 1999). They are also present in vegetable oils and partially hydrogenated fats in low concentration (Banni et al. 1994).

The CLA is a rather complex mixture, Sehat et al. (1998) identified twenty different isomers in cheese. According to Banni and Martin (1998) the principal isomer in dairy products is the (9Z,11E)-octadecadienoic acid accounting for 80-90% of the total CLA. In contrast, its proportion in vegetable oils is less than 50%. CLA preparations commercially available as dietary supplements typically contain about 35% (9Z,11E)-isomer (Kramer et al. 1998). These products are obtained by alkaline isomerization of linoleate-rich oils and may contain a wide range of isomers.

2.3. REFINING OF VEGETABLE OILS

The worldwide production of the four major vegetable oils accounted for more than 71 million tons in 2000. Soybean oil and palm oil represented 25.5 and 21.8 million tons respectively, the rapeseed oil production was 14.3 million tons, that of the sunflower oil 9.7 million tons (Oil World Annual 2001).

To process such volumes, huge capacity increasingly automated refining lines are

worldwide operated. During the operation, various minor components often referred to as impurities are removed from crude oils. The undesirable components of crude vegetable oils and the two major alternative refining methods will be briefly overviewed below. Deodorization will be discussed in chapter 2.4.

2.3.1. Classification of impurities in crude oils

According to the classification by Hebendanz (1990), “impurities” in vegetable oils have three major sources. Firstly, they can be minor components, naturally present in the oil such as phospholipids, waxes and pigments. The quantity and composition of these compounds are typical to a given oil.

The second group includes diverse substances formed by the degradation of the oils’ natural constituents (non-hydratable phospholipids, free fatty acids, peroxides, aldehydes, ketones). These compounds develop in the seed or in the oil during storage and processing.

The third type of impurities comprises residues of chemicals used in seed growing or processing, their derivatives and contaminants from the applied equipment. This group includes pesticides, polycyclic aromatic hydrocarbons, solvent residues, metal traces, soaps, phosphoric and citric acid.

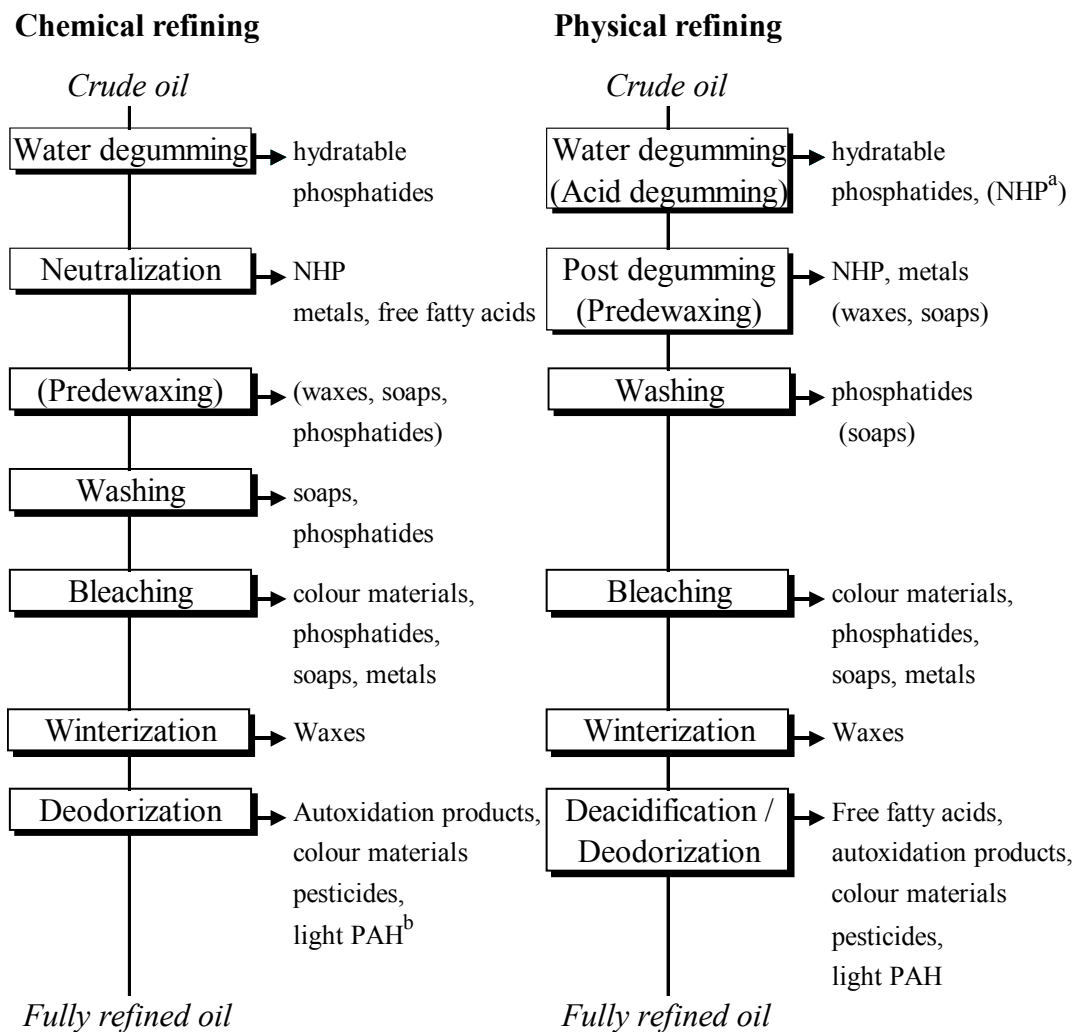
Attention has to be paid to the storage and handling of oilseeds and oils in order to minimize the formation of undesirable components. Refining processes comprise the consecutive steps performed to remove these substances from crude oils.

2.3.2. Refining methods

The edible oil processors apply two principal processes. The traditional way to get rid of impurities listed above is the chemical refining, in which caustic soda is used to remove free fatty acids in form of soaps. In the other worldwide-applied process,

called physical refining, free fatty acids are stripped from the oil during deodorization at high temperature under reduced pressure. Figure 8 outlines the main steps of the two processes.

Figure 8
Overview of refining processes



^a non-hydratable phospholipides

^b polycyclic aromatic hydrocarbons

2.3.2.1. Degumming

Degumming processes refer to the removal of phospholipids, representing 0.5-3.0%

of crude oils. In presence of water, they form a precipitate in the oil, which does not meet the consumers' preferences. Their substantial quantity in refined oils makes the taste unacceptable during a short storage period. Phospholipids are often linked to prooxidative heavy metals. The incomplete removal of phospholipids increases the losses during neutralization and results in difficulties in bleaching by inactivating the bleaching earth and blocking the filters.

Water degumming is the simplest method to reduce phosphatide content. Soft water is used for hydration taking place during 20-30 minutes at 80-85°C, the gums are then separated from the oil based on their increased density by centrifuging. As only the hydratable phosphatides can be removed with this technique, the residual phosphorus content of the water degummed oil is typically 80-200 mg/kg (Gibon and Tirtiaux 2000).

Acid degumming provides a more complete phospholipid elimination by using mineral or organic acid to convert the non-hydratable phosphatide salts into hydratable form. Phosphoric or citric acid is most frequently applied. After the acid treatment and hydration, the gums are separated as described above.

In case of physical refining the efficient phospholipid elimination before deacidification-deodorization has a special importance. For this reason a great number of post-degumming methods have been developed (Dijkstra 1998). Applied after water- or acid degumming these methods provide less than 10 mg/kg residual phosphorus in the oil.

2.3.2.2. Neutralization

Free fatty acids have a negative impact on the taste and oxidative stability of the refined oil. Traditionally, they are eliminated by caustic soda treatment in form of soaps known as soapstock. Beside the free fatty acid elimination, the removal of phosphatides is completed and parallel a part of the pigments is destroyed. To complete the neutralization reaction the caustic soda is used in a slight excess. The

concentration of the applied sodium-hydroxide solution ranges between 65 and 235 g/l in general. The exact concentration and the excess amount is determined by the acidity, the oxidative state and the nature of pigments in the oil (Hodgson 1996).

In the Short-Mix process of Alfa Laval, preferred in Europe, the contact between the oil and caustic soda is limited to 3-10 seconds at 85°C. In this way neutralization is completed without significant risk of secondary saponification (reaction of triacylglycerols with sodium-hydroxide). The majority of the soaps formed during the neutralization reaction are removed on a primary centrifuge. The residual soaps are removed in one or two washing steps performed at around 90°C applying 6-8% hot water in the first, 4-7% in the second stage. The oil loss arising during neutralization has two sources. The unavoidable loss corresponds to the quantity of free fatty acids and other impurities removed. The neutral oil loss measures the neutral oil entrained in the soapstock and washing water and to the oil lost by secondary saponification in case of a not properly performed process.

In case of sunflower oil the first washing (and similarly the post-degumming in case of physical refining) is often combined with the removal of the major part of the waxes, the operation is named predewaxing. The process version of Westfalia company consist of a crystallization step at 4°C for 6 hours in presence of 400-800 ppm of soap and 5-7% water and a cold centrifugation. The wax content of the predewaxed oil is typically lower than 150 mg/kg.

2.3.2.3. Bleaching

Bleaching of oils is based on an adsorption process that removes color bodies and other minor impurities like traces of soaps, phospholipids and heavy metals. Over its decolorizing effect, bleaching has a great influence on the taste and oxidative stability of deodorized oil. During bleaching hydroperoxides, present as primary products of oxidation of the oil decompose and various volatile compounds including aldehydes and ketones appear. A wide range of bleaching agents such as

natural (non-activated) clays, acid activated bleaching earth, activated carbon and synthetic silica hydrogels are used as adsorbent. Most commonly 0.05-0.2% activated earth is used for sunflower, 0.4-0.7% for corn and 0.6-0.9% for soya and rapeseed oils (Denise 1983).

Bleaching is performed in continuous vacuum bleachers. The oil mixed previously with the adsorbent and filter aid is deaerated in the upper part of the equipment and heated up to the bleaching temperature. The adsorption is then completed in the bleaching section. It is essential to provide an intimate contact between the oil and the adsorbent as well as a sufficient contact time (20-30 min) and an adequate temperature (90-110°C) to obtain efficient adsorption. In order to avoid undesirable side reactions and the loss of tocopherols, reduced pressure (4-8 kPa) is applied. The bleached oil and the used bleaching earth are separated in hermetically closed filters, provided for alternate use. Filter aid is employed in the process to facilitate filtration.

2.3.2.4. Winterization

Winterization aims at the removal of high-melting-point substances (referred as to waxes). Making the oil haze and forming deposit, waxes have negative affect on the appearance of the product. Their removal consists of a cooling, crystallization and filtration step.

After cooling, filter aid is introduced into the oil proportionally to the wax content. Waxes are than allowed to crystallize in a series of chilled, gently agitated tanks. Finally, the waxy cake is removed on a leaf filter. In order to facilitate the filtration by decreasing the viscosity, the oil is slightly preheated before passing to the filter.

2.4. DEODORIZATION PROCESSES

Deodorization is the final stage of refining processes, aiming at the removal of

odoriferous materials and flavor substances characteristic to the nature of the oil. These undesirable substances are evaporated at high temperature (180-260°C) under reduced pressure (0.1-1.0 kPa) with steam or nitrogen stripping. During deodorization free fatty acids, and lipid oxidation products are removed, the color of the oil turns lighter (heat bleaching) and some contaminants such as pesticides and herbicides light polycyclic aromatic hydrocarbons are also eliminated. The removed materials are recovered in the deodorization distillate, the composition of which depends on the type of oil and the applied process as demonstrated in Table 5. Deodorization distillates receive increasing interest as natural sources of numerous valuable compounds: plant sterols, tocopherols and phytosqualene.

Table 5
Typical composition of deodorization distillates

Refining	Oil type	Free fatty acids %	Unsaponifiable material, %	Tocopherols %	Sterols %
Chemical	Soy	30-40	25-33	6.0-12.0	6.0-13.0
Chemical	Sunflower	30-50	27-32	4.0-5.0	6.0-9.0
Chemical	Rape	30-50	28-35	4.5-5.5	7.0-11.0
Physical	Sunflower	60-85	10-12	1.0-2.0	2.5-3.5
Physical	Rape	60-85	8-11	1.5-2.5	3.0-5.0

The completeness of deodorization is indicated by the residual FFA content of the oil (typically <0.06%) and the peroxide value (practically 0 meq/kg at the outlet of the deodorizer). From the point of view of organoleptic properties, a properly deodorized oil has neutral taste and odor. Under the deodorization conditions numerous side-reactions may also take place including geometrical isomerisation of fatty acids, formation of triglyceride dimers and trimers, hydrolysis by the stripping steam, re-esterification, color fixation and formation of off-flavors (Dijkstra 1995a). Contrary to the chemical refining, in which only little amount of free fatty acids is eliminated during deodorization, the physical refining process combines

deodorization and deacidification into one step referred as to “deacidification-deodorization” or “physical refining”.

In principle today most deodorizers are designed to enable physical refining, with other words to strip higher quantity of free fatty acids from the oil. Compared to chemical refining to handle the additional vapor from the evaporation of free fatty acids and to maintain a lower operating pressure, physical refining requires larger vacuum system. Moreover, it needs higher quantity of stripping steam. On the other hand physical refining has many advantages from the point of view of operating costs, investment need and environmental aspects, which makes the process very attractive to apply in new installations (Segers 1985). As free fatty acids are recovered in the deodorization distillate, no soapstock forms in physical refining, consequently there is no need for the associated waste water treatment and this also leads to a lower environmental load. Using fewer centrifuges, the investment need of the physical process is lower than that characteristic to chemical refining. Concerning the applicability of the two processes, chemical refining is less sensitive to the quality of crude oil.

2.4.1. Theoretical background

The basis of deodorization is the large volatility difference between triacylglycerols and the undesirable components present in low concentrations. Having much higher vapor pressure than triacylglycerols, these substances can be distilled from the oil at high temperature and low pressure with the aid of stripping steam. The theoretical background of steam distillation is described in several publications (Bailey 1941, Athanassiadis 1988). A summary of the calculation of stripping steam requirement derived by Bailey is given as follows.

Assuming that the system consisting of the oil and volatile impurities conforms to Raoult’s law, the vapor pressures of the volatile compounds at a given temperature

can be expressed by the form below:

$$p_v = \frac{P_v \cdot n_v}{n_o + n_v} \quad \text{Equation 1}$$

where

p_v is equilibrium vapor pressure of a given volatile compound,

P_v is vapor pressure of the pure volatile compound,

n_v is the number of moles of the volatile compound and

n_o is the number of moles of the oil.

Except for an oil of high free fatty acid content n_v is very small related to n_o , consequently equation (1) simplifies:

$$p_v = \frac{P_v \cdot n_v}{n_o} \quad \text{Equation 2}$$

Applying Dalton's law the molecular ratio of volatile materials and steam in the vapour phase equals to the ratio of their partial pressures.

$$\frac{dn_s}{dn_v} = \frac{p_s}{p_{v'}} \quad \text{Equation 3}$$

where

n_s is the number of moles of steam,

p_s is partial pressure of steam and

$p_{v'}$ is partial pressure of the volatile compound

As $p_{v'}$ is very small compared to p_s , this later closely approaches the total pressure, P :

$$\frac{dn_s}{dn_v} = \frac{P}{p_{v'}} \quad \text{Equation 4}$$

In case of ideal mixing of the stripping steam and the oil, the partial pressure of a given volatile compound is the same in the vapor and liquid phase. As the ideal case is not reached in the practice, therefore a factor of vaporization efficiency, E can be introduced:

$$E = \frac{p_{v'}}{p_v} \quad \text{Equation 5}$$

This factor measures the degree, with which the stripping steam becomes saturated when passing through the oil. It becomes from Whitman's two-film theory of gas absorption that at any instant the transfer rate of volatile materials from the oil into the steam bubble is equal to the difference between the saturation pressure in the bubble and the actual pressure, multiplied with the surface area of the bubble and a constant characteristic to the oil and the steam.

Mathematically,

$$\frac{dp_{v'}}{dt} = k \cdot A \cdot (p_v - p_{v'}) \quad \text{Equation 6}$$

where

t is the contact time between the steam bubble and the oil,

A is the surface area of the steam bubble and

k is the constant of gas diffusion.

Integrating equation (6) and applying equation (5):

$$A \cdot k \cdot t = \ln \frac{p_v}{p_v - p_{v'}} = \ln \frac{1}{1 - E} \quad \text{Equation 7}$$

or,

$$E = 1 - e^{-A \cdot k \cdot t} \quad \text{Equation 8}$$

It follows from equation (8) that the vaporization efficiency increases with an

extension of the total surface area of the steam bubble and the contact time between the steam bubble and oil. From practical approach, the efficiency factor can be influenced by the geometry of steam injection and the depth of the oil layer. In a too shallow oil layer there is an increased risk that the steam reaches the surface without a sufficient level of saturation. On the other side the contact time is lengthened in a deep layer but the arising agitation problems lead to an inadequate renewal of the oil surface and a non-uniform treatment of the oil in this case.

The basic influence of the deodorizer design on the vaporization efficiency was shown by Deffense (1993) at laboratory scale. Using a glass equipment, elimination of reflux and radiation losses and optimization of the steam distributor device reduced the steam consumption from 1.5% to 0.55% and increased the efficiency factor from 0.42 to 0.93.

To express the steam quantity necessary for the distillation equations (2), (5) and (4) are combined:

$$\frac{dn_s}{dn_v} = \frac{P \cdot n_o}{E \cdot P_v \cdot n_v} \quad \text{Equation 9}$$

By integration of equation (9):

$$n_s = \frac{P \cdot n_o}{E \cdot P_v} \cdot \ln \frac{n_{v1}}{n_{v2}} \quad \text{Equation 10}$$

where

n_{v1} is the number of moles of the volatile component in the oil before deodorization, n_{v2} is the number of moles of the volatile component in the oil after deodorization.

Equation (10) expresses that the quantity of steam needed for deodorization is directly proportional to the oil quantity and the absolute pressure in the deodorizer and inversely proportional to the vapor pressure of the pure volatile compound at the applied temperature. It is also inversely proportional to the vaporization efficiency, meaning if it is poor more steam is required.

In equation (10) ideal solutions are assumed. However, mixtures of vegetable oil and fatty acids differ from ideal. Therefore, Szabó Sarkadi (1958) introduced an α activity coefficient, which gives finally:

$$n_s = \frac{P \cdot n_o}{E \cdot P_v \cdot \alpha} \cdot \ln \frac{n_{v1}}{n_{v2}} \quad \text{Equation 11}$$

Without the simplifications applied in equation (2) and (4), the steam requirement can be expressed by the full Bailey equation (Deffense 1995):

$$n_s = \frac{P \cdot n_o}{E \cdot P_v} \cdot \ln \frac{n_{v1}}{n_{v2}} + \frac{P}{E \cdot P_v} \cdot (n_{v1} - n_{v2}) - (n_{v1} - n_{v2}) \quad \text{Equation 12}$$

The right hand side of equation (12) consists of three terms, the first part of which is the simplified Bailey equation (10). The middle term originates from the simplification introduced in equation (2), assuming that the number of moles of volatile substances can be neglected compared to that of the oil. In case of chemical refining this simplification is well founded and even in case of physical refining it makes a relatively small error as demonstrated by Dijkstra (1999b). The last part derives from the simplification used in equation (4), where it was assumed that the partial pressure of the volatile compound is very small compared to the total pressure. This is justified for chemical refining, but in case of physical refining, especially during its early stages the partial pressure of the free fatty acids is not negligible. Skipping the third part of equation (12) leads to a considerable overestimation of the steam requirement (Deffense 1995), consequently for the physical refining process the full equation (12) have to be applied.

2.4.2. Influence of the operating parameters

The four main parameters influencing the deodorization are the time and

temperature of the operation, the pressure and the rate of stripping gas.

Temperature and time.

The Clausius-Clapeyron equation describes the relation between the vapor pressure of a volatile compound and the absolute temperature. In the practice, the vapor pressure of fatty acids at different temperatures is calculated by Lederer's empirical formule. It follows from both equations that the evaporation of the volatile substances is faster with increasing temperature. Consequently, the deodorization time and the size of the deodorizer can be decreased. As an approximation, for each 20°C increase in temperature reduces the time necessary to distill free fatty acids by half (Dudrow 1983).

It can be derived from equation (10) that the necessary amount of steam decreases with increasing temperature. Reduction of stripping steam consumption is an important factor in reducing the neutral oil loss.

The higher process temperature results in a so-called heat bleaching effect, which comprises the thermal decomposition of peroxides and pigments, necessary to obtain refined oil of good stability. On the other hand, excessive temperatures have to be avoided because of the possible geometrical isomerization of polyunsaturated fatty acids and the formation of polymerized triacylglycerols. However, being more saturated, palm oil is generally deodorized at elevated temperature because a maximum heat bleaching effect is desired.

With the increasing temperature, the evaporation loss of some valuable minor components such as tocopherols and sterols rapidly increases. The decrease in tocopherol content is mainly a distillation effect and not a consequence of thermal breakdown (De Greyt 1997).

There are important differences concerning the deodorization practice in the United States and in Europe. In the U.S. soybean oil is deodorized at high temperature (240-260°C) during short time (30-60 min) to increase the capacity or to decrease the size of the equipment to be installed. According to the European practice, 220-

250°C is the general range of operating temperatures.

Pressure and stripping gas.

It derives from equation (10) that the stripping steam required is directly proportional to the operating pressure. Therefore, a lower pressure will allow a lower stripping steam rate (Zehnder 1975). The operating pressure is limited by the cost of the equipment. A high rate of stripping steam increases the hydrolysis and the oil loss by mechanical entrainment. As a compromise between the lowest possible pressure, the cost of the vacuum system and the operating cost deodorizers are operated most frequently between 0.2 and 0.5 kPa.

Instead of steam, an inert gas such as nitrogen can also be applied as an environmental friendly alternative. Its use in oil refining depends mainly on the cost of nitrogen and as nitrogen is a non-condensable gas, on the cost of the larger vacuum system (Constante et al. 1994).

2.4.3. Deodorizer design

The performance of deodorization system strongly depends on numerous factors that have to be considered at design level.

2.4.3.1. Construction material

Due to its prooxidant effect, carbon steel is not used for building deodorizers. Being strong prooxidant it must be avoided that copper gets in contact with the oil (Carlson 1996). In physical refining the equipment is exposed to corrosive fatty acids, that is why increasingly acid resistant stainless steel is preferred to stainless steel for all parts of the deodorizer that is in contact with the oil or vapors.

In order to prevent the oil from oxidation air leakages must be minimized. At the temperature of deodorization, leaks sufficient to spoil the oil flavor may not be enough to reduce the vacuum and so they can be hard to detect by testing.

2.4.3.2. Heating requirement

The net heating energy requirement for a deodorization system is a function of the specific heat capacity of the oil, the heat loss from radiation and the heat recovery. The heat recovery mainly depends on the type of deodorization system. For a given installation, it can be maximized by optimizing the heat transfer conditions. This is supported by the latest types of external and internal heat exchangers designed for heat recovery. Generally, better recovery can be achieved with external heat exchangers and they are easier to clean. On the other hand, internal heat exchangers are necessary for heat recovery under vacuum and ensure less intermixing. The heat recovery is performed in two steps in most cases. The appropriate heat exchangers are selected taking into account their thermal performance, maintenance need, level of intermixing. It is also a basic criterion to have no adverse effect on the oil quality (Athassiadis 1988). The most widely used external heat exchangers are plate, spiral and shell-and-tube designed.

Concerning heating medium, generally high-pressure steam is applied in the final heating step to reach the operating temperature. Investing in a high-pressure steam boiler increases the equipment cost and in the past mineral-based heating fluids provided an inexpensive alternative. However, application of such medium was abandoned because of the risk of product contamination (Meershoek 1998).

2.4.3.4. Vacuum generation

The low absolute pressure (0.1-1.0 kPa) applied for deodorization is generated by vacuum systems, consisting of a combination of steam-jet ejectors, vapor condensers and mechanical vacuum pumps. In industrial installations, three-stage steam-jet ejectors (boosters) are widely used, which provide 0.5-0.8 kPa. If lower pressure is requested a four-stage steam-jet ejector is used or a mechanical (liquid ring) vacuum pump is applied in the final stage.

The utilities for driving the vacuum system are motive steam, coolant and

electricity. They increase with the vapor load, degree of vacuum and the temperature of coolant. An efficient way of economizing on these utilities is to reduce the vapor load. The vapors consist of various volatile impurities, fatty acids, entrained oil, steam and non-condensable gases (air). A fatty material condenser (scrubber) is generally installed preceding the vacuum system to recover the fatty substances and to decrease the effluents. For this reason, the vapors are passed through a packed tower or a spray condenser, where the volatile materials and the entrained oil are cooled and condensed by contacting the recycled distillate. The typical temperature of the recycled material is 55-65°C, at which efficient fatty acid recovery is obtained without condensing any steam. The excess distillate is regularly discharged into a deodorization distillate tank.

After the fatty matter condenser, the vapor load is composed mainly of steam and non-condensables. The stripping steam amount is inversely proportional to the absolute pressure, the quantity of non-condensable gases is determined by the air tightness of the system.

The energy consumption for generating vacuum can be efficiently decreased by decreasing the stripping steam. Stripping steam consumption decreases with increasing temperature and vacuum. Kellens (1997) demonstrated that a 53.3% saving can be obtained by increasing the temperature of deodorization from 240°C to 260°C and decreasing the pressure from 0.3 kPa to 0.2 kPa simultaneously.

The portion of steam in the vapor load can be greatly reduced by using a total condensing system, in which stripping steam is condensed. In this way, less motive steam is demanded for vacuum generation and the emission of odoriferous materials is reduced.

2.4.3.5. Stripping medium requirement

The necessary amount of stripping agent increases with its molecular weight. Consequently, the possible lowest molecular weight stripping medium is selected.

From economical point of view, most frequently steam is used. In some cases, however, nitrogen is preferred. Being an inert and non-condensable gas, nitrogen stripping results in lower losses by eliminating the hydrolysis and lowering the entrainment. Therefore, as Constante et al. (1991) pointed out, the deodorization distillate has better quality. Comparing to the use of stripping steam, the price of nitrogen is higher and the vacuum system has to be adapted in order to maintain the same operating pressure.

The stripping medium has to be dry and free of oxygen. In case of steam the feed water has to be deaerated, attention has to be paid to avoid carryover of boiler water solids. Superheating ensures that the stripping agent is dry and no cooling of the oil occurs.

2.4.4. Industrial deodorizer systems

Several types of industrial deodorizer are available. Factors including the frequency of feed change, heat recovery, the amount of investment and operating costs have to be considered when selecting the most appropriate system.

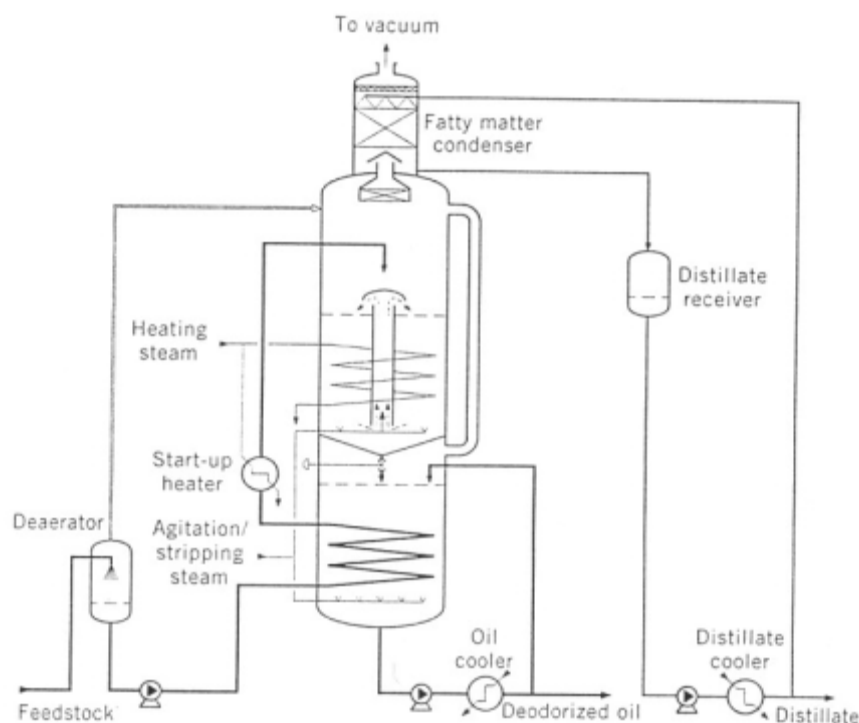
2.4.4.1. Batch deodorizers

Applying batch deodorization, all steps of the operation such as deaeration, heating, deodorization, and cooling are performed in the same vessel (Figure 9). Batch systems are used for low capacity plants (<100 tons/day), processing different oils with the demand of minimal intermixing. The high steam consumption, the low degree of heat recovery and the high peak demand on heating steam and cooling water are the main disadvantages of the process.

Batch deodorizers traditionally consist of a single-shell vertical cylindrical vessel of welded construction. Its total capacity is at least double of the batch in order to reduce the neutral oil loss provoked by the intensive splashing during the operation.

This carryover is further minimised by an entrainment separator positioned ahead of the vapour outlet. The stripping steam is usually injected in a gas lift tube, also referred as to mammoth pump. This solution provides an efficient mixing as well. Heating and cooling is provided by internal pipe coils or by external heat exchangers. Internal coils provide a better heat recovery (up to 50 %) and a lower intermixing of different feed stocks.

Figure 9
Batch deodorizer (Carlson 1996)



2.4.4.2. Semi-continuous deodorizers

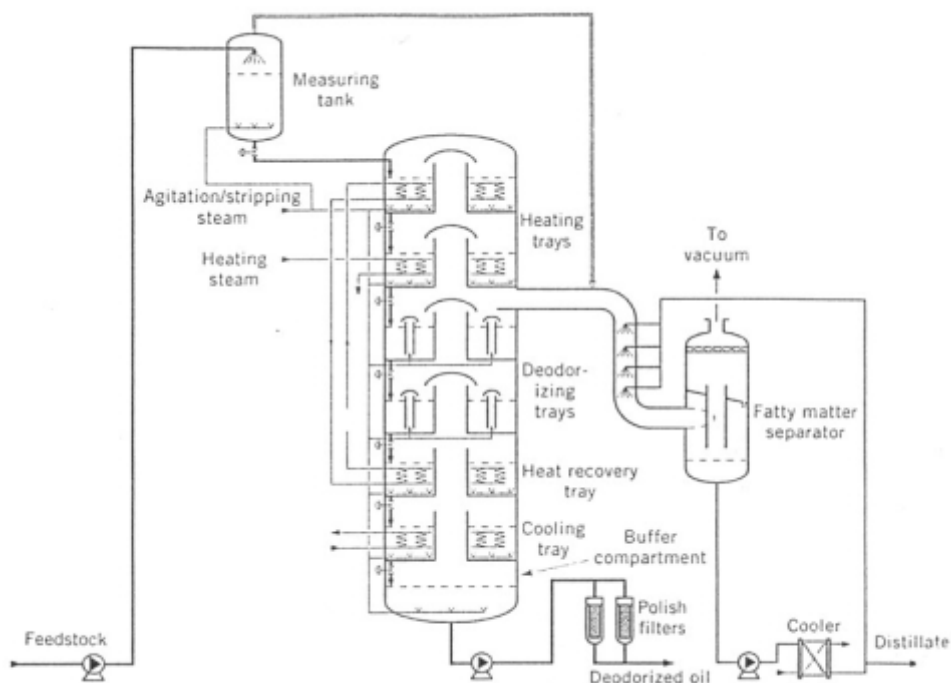
These installations are batch systems designed for higher capacities. The semi-continuous process permits frequent stock changes with practically no intermixing. Compared to the continuous deodorization the required time to change the feed stock is shorter and the quantity of residual oil in the system is smaller. The disadvantages of semi-continuous deodorization are the high investment and

operating costs and the lower heat recovery (40-50%).

As shown in Figure 10, the oil batch is transferred by the gravity through a number of vertically stacked compartments or trays. The oil is progressively deaerated, heated in the first tray, deodorized in the middle sections and cooled in the last tray under vacuum. Pipe distributors or mammoth pumps are used for sparging steam injection. The steam injected in the heating and cooling trays provides agitation.

Figure 10

Semi-continuous deodorizer, De Smet (Carlson 1996)



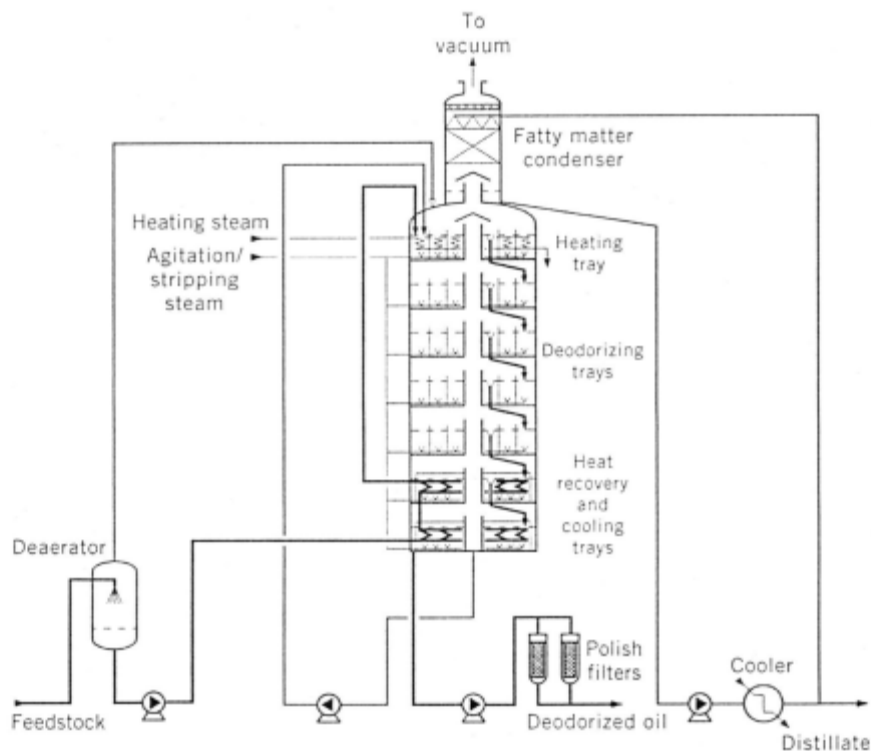
2.4.4.3. Continuous deodorizers

For high capacity plants with few stock changes continuous deodorizers are the most widely applied. The relatively low investment need and the high heat recovery are their main advantages. Most designs consist of a series of steam-agitated trays placed in a cylindrical shell (Figure 11a.). In order to obtain a plug flow, the flow is

channelled inside the trays by vertical baffles. Stripping gas is injected at each tray by pipe distributors or gas lift pumps arranged between the baffles. The liquid level on the trays is maintained by overflow pipes.

In continuous deodorizers high heat recovery can be obtained, the degree of which depends on the sensitivity of the oil to heating and cooling under vacuum. Palm oil and lauric types can be fully heated up and cooled down in external heat exchangers resulting in a heat recovery up to 80%. More unsaturated oils such as rapeseed or soybean oils require partial cooling under vacuum to avoid flavour problems, which makes heat recovery more difficult.

Figure 11a.
Continuous deodorizer, Krupp (Carlson 1996)



2.4.4.4. Thin film deodorizers

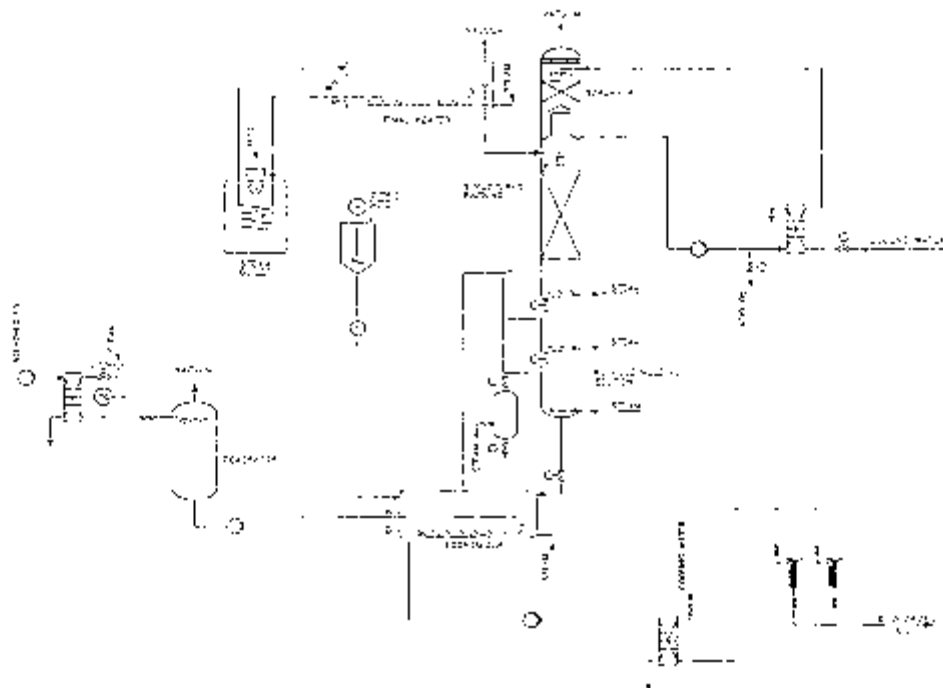
Thin film deodorization provides an economic and flexible process alternative for refining of vegetable oils (Stenberg 1996 and Ahrens 1999). Thin film or packed

column deodorizers provide high capacities, low operating cost, high heat recovery (up to 80%) and very small entrainment loss. On the other side the degree of feedstock intermixing is high, the system is very sensitive to air leakage and to the fouling of the packing caused by polymeric compounds formed at higher temperatures.

In case of packed column deodorization the deaerated and heated oil flows by the gravity over the packed section agitated with stripping steam. Structured packing is applied, which enables an even oil distribution and avoids local overheating. Thanks to the very high surface area, free fatty acids and other volatile compounds are removed during a very short time, which is not sufficient for an efficient heat bleaching and deodorization. Therefore, a tray-type holding section is added, where the retention time is adjusted by the oil level in the trays.

Figure 11b.

Soft column deodorizer, Alfa Laval (Ahrens 1999)



Adjustment of captions

Please do not delete this section

Figure 12 Figure 13 Figure 14 Figure 15 Figure 16 Figure 17 Figure 18 Figure 19 Figure 20 Figure
21 Figure 22

Table 6 Table 7 Table 8 Table 9 Table 10

Equation 13 Equation 14 Equation 15 Equation 16 Equation 17 Equation 18 Equation 19 Equation
20 Equation 21 Equation 22 Equation 23 Equation 24

3. EXPERIMENTAL

3.1. MATERIALS AND METHODS

3.1.1. Materials

The different oils used as raw material for the experiments were identified by capitalized letters (A-H). The experiments were signified with numbers (1-11). Rapeseed oils “A-F” for the laboratory experiments (1-8) were supplied by the Cereol refinery located in Coudekerque-Branche (France). Pilot plant scale experiments (9-11) were carried out on UF degummed bleached rapeseed oil “G” provided by Cereol Martfű refinery (Hungary).

Raisio Margariini (Raisio, Finland) supplied neutralized bleached rapeseed oil “H” to produce specially treated oil for nutritional studies at pilot plant scale (chapter 4.3.2.).

To check the influence of deodorizer design (chapter 4.3.3.), refined sunflower, rape and soybean oils were experimentally produced at different European refineries of Cereol Group.

For the survey of (*E*) fatty acid content of commercially available refined oils (chapter 4.4.), different bottled oil products were bought in supermarkets in Austria, Germany, Hungary, Poland, Romania, and in the United States.

Analytical grade solvents and reagents (Reanal Hungary, Merck Germany and Sigma Chemicals Hungary) were used for all measurements.

3.1.2. Experimental procedures

Besides its original meaning, the term of deodorization will be used in this study denominating special prolonged heating of vegetable oils under vacuum.

Nevertheless, this operation is not to be confused with the regular vacuum-steam deodorization.

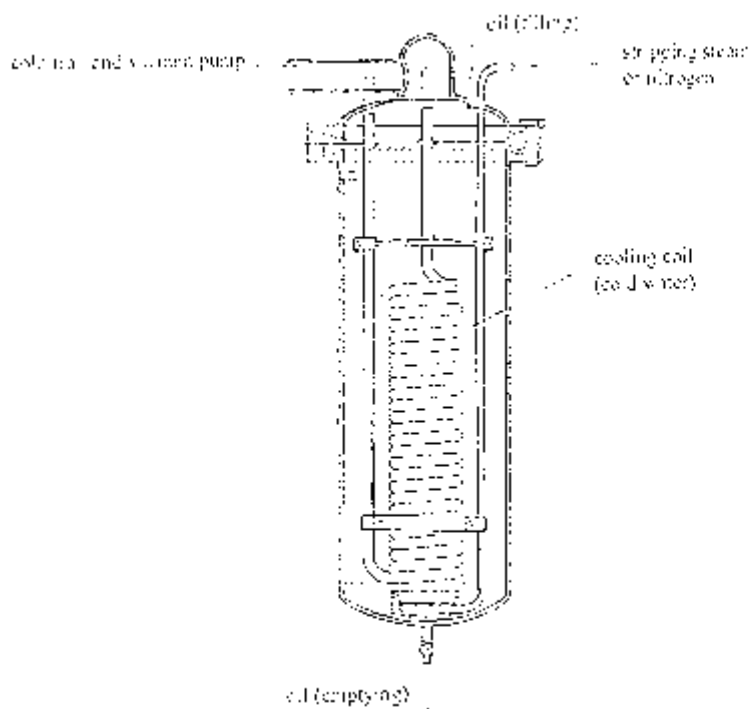
Laboratory experiments were performed at the Cereol Group Center of Expertise, Cappelle la Grande (formerly Lesieur Recherche et Développement), France. Pilot plant scale trials were accomplished at the Cereol Group Center of Expertise, Budapest, Hungary.

Laboratory scale deodorization

The experiments were carried out using a home made deodorizer having a total capacity of 3.2 liters (Figure 23). The stripping gas, steam or nitrogen was injected into the oil through small holes arranged in the sparger plate at the bottom of the equipment. The oil was introduced into the deodorizer under vacuum and heated by means of an external electric jacket. About 20 minutes was needed to reach a final temperature of 200°C. The overheating before the oil temperature could be stabilized was less than 10°C and lasted not longer than 10 minutes. The operating temperature was kept within $\pm 1^\circ\text{C}$. In order to avoid water condensation inside the deodorizer, nitrogen stripping was always used at the beginning and at the end of the operation when the temperature was below 120°C. The pressure in the equipment was less than 0.4 kPa during the operation.

The residence time was started when the deodorization temperature was reached. In some cases the experiment was completed without interruption (continuous operation). When it was necessary to interrupt a long-term experiment at the end of the day (discontinuous operation), the oil was cooled down with cold water flowing through a coil placed inside the deodorizer. The cold oil was kept in the equipment under a positive nitrogen pressure until the next deodorization step. In this way, the deodorization time was the cumulative time of the consecutive steps. Samples for analysis were taken during the experiments by applying a nitrogen overpressure inside the equipment before opening the sampling valve. The hot sample was quickly cooled down and stored in refrigerator in a tightly closed vial until analysis.

Figure 12
Laboratory scale deodorizer



Pilot plant scale deodorization

Pilot plant scale experiments were carried out in a 100-liter batch deodorizer (Figure 24). The oil was heated up to the operating temperature under reduced pressure. Taking into account the batch size and the extremely long duration of the tests, it was more convenient to apply nitrogen for agitation and stripping during the whole operation. Nitrogen was injected at the bottom of the equipment by means of a sparger plate. A hot silicon liquid, Syltherm 800 was circulated in the equipment jacket for heating. Approximately 1 hour was necessary to raise the oil temperature to 200°C. The residence time was counted after reaching the desired temperature. The accuracy of the temperature regulation was $\pm 1^\circ\text{C}$. During the operation the pressure was maintained at 0.2 kPa. The pilot plant scale experiments were performed in continuous way, in 12-hour shifts. After reaching the operating

temperature, the oil was regularly sampled through a cooling sampling system. The samples were taken out at room temperature and stored in refrigerator in a tightly closed vessel until analysis.

Figure 13
Pilot plant batch deodorizer



3.1.3. Analytical methods

3.1.3.1. Analysis of fatty acid composition

Method 1.

The samples of the laboratory experiments (chapter 4.1.) were analyzed according to the method used routinely at the Cereol Group Center of Expertise, Capelle la Grande.

Fatty acid methyl esters (FAME) were prepared according to the American Oil Chemists' Society Official Method (1993a), AOCS Ce 2-66. Glycerides were saponified with methanolic sodium-hydroxide, the soaps were converted into methyl esters by reaction with a boron trifluoride/methanol complex.

An FFAP fused silica capillary column 25m x 0.2 mm i.d., 0.33 μm film thickness (Hewlett-Packard, Les Ulis, France) was used for FAME analysis. The carrier gas was hydrogen at a flow rate of 1.2 mL/min. The samples were analyzed on a HP5890 Series II gas chromatograph (Hewlett Packard-Palo Alto-CA). The split/splitless injector was set to 300°C, the flame ionization detector was heated to 250°C. The oven temperature was programmed from 170°C to 190°C at 1°C/min, then to 220°C at 2°C/min followed by a 14 min hold.

The gas chromatograph was calibrated using a FAME mixture (Alltech Templeuve, France) containing 16:0, 18:0, 18:1, 18:2, 18:3 FAME in equal amount. The (*Z*) and (*E*) geometrical acid isomers were identified by reference to literature (Wolff 1993b).

Method 2.

The samples collected during pilot plant deodorization (chapter 4.2 and 4.3.2.), the industrial samples (chapter 4.3.3.) and the commercial refined oils (chapter 4.4.) were analyzed by the method routinely applied at the Cereol Group Center of Expertise, Budapest.

Fatty acid methyl esters (FAME) were prepared according to the American Oil Chemists' Society Official Method (1993a), AOCS Ce 2-66. FAME were analyzed on a 5890 Series II plus gas chromatograph (Hewlett Packard) equipped with an electronic pressure control system, a split/splitless injector (210°C) and a flame ionization detector set at 210°C (Attachment 1). A fused silica column 60 m x 0.25 mm ID, 0.2 μm film coated with SP2340 (Supelco, Inc., Bellafonte, PA) was used for FAME analysis. The carrier gas was hydrogen at a constant flow rate of 20

cm/sec. The oven temperature was programmed from 150°C to 200°C at 1.3 °C/min, then held at 200°C for 10 min.

The equipment was calibrated and the geometrical isomers were identified as described in *method 1*.

Before the experiments *method 1* and *method 2* were cross-checked in an internal survey. Fatty acid composition of two test samples were analysed by both methods (2-2 parallel measurements), the mean values were compared using the Student's t-test. No significant difference was found at a confidence level of 95%.

3.1.3.2. Other analysis

As the oils produced in chapter 3.4.2. were used in a separate nutritional study on humans, a more detailed characterization of these products was accomplished.

Tocopherols

Tocopherol content was measured according to the ISO 9936: 1997 method, using HPLC (Waters 600E Multisolvent Delivery System equipped with Hewlett-Packard FLD-1046A fluorescence detector).

Peroxide value

Determination of peroxide value was performed by the ISO 3960: 1997 method, using iodometric titration.

Free fatty acid content

Acidity of the oils was determined according to the ISO 990: 1996 method, applying acid-base titration. Free fatty acid content was expressed as % oleic acid.

Light transmittance at 420 nm

The transmittance value at 420 nm was measured by an internal spectrophotometric method using a Hewlett-Packard 8452A UV-VIS spectrophotometer.

Sensory evaluation

Sensory characteristics were determined based on the American Oil Chemists' Society Official Method (1993b), AOCS Cg 2-83, 3-4 panellists evaluated the taste

and odour of deodorized oils.

3.1.3.3. Statistical evaluation of the results

The reliability of the laboratory model (concerning total linolenic acid content, (*E*)-linoleic acid content and (*E*)-linolenic acid content) was checked by two statistical tests. The differences between the values measured in the pilot plant experiments and those calculated using the theoretical model were analyzed. The Student's t-test was applied to determine the significance of these differences. The Abbe test was conducted to investigate whether there was a systematic order in the differences (S. Kemény and Deák 1999). The critical values at a confidence level of 95% and the calculated values of the test statistics were summarized in tabulated form.

4. RESULTS AND DISCUSSION

4.1. LABORATORY SCALE MODELING OF GEOMETRICAL ISOMERIZATION OF POLYUNSATURATED FATTY ACIDS

Laboratory experiments were carried out aiming at determination of rate constants describing the isomerization of linoleic and linolenic acid during prolonged heating. First, the degradation of linoleic and linolenic acid was determined, which was then taken into account when determining the isomerization rate constants.

The neutralized bleached rapeseed oil used in the tests contained 20.4-21.5% linoleic acid and 8.4-9.7% linolenic acid, except for experiment 6, where an already deodorized rapeseed oil was re-deodorized in the laboratory (Table 11). No appreciable amount of (*E*)-isomers of oleic and linoleic acids and a very small amount of (*E*)-linolenic acid (not more than 0.06%) was found in the bleached oils. The starting deodorized oil used in experiment 6 contained 0.4% (*E*)-linoleic and 1.26% of (*E*)-linolenic acid. Tests were carried out according to the procedure described in chapter 3.1.2. at temperatures ranging from 210 to 270°C over 2 to 65 hours (Table 12).

Table 6
Fatty acid composition (%) of rapeseed oils used in the laboratory experiments

Oil id.	A	B	C	D	E	F
Experiment no.	1 ^a	2 ^a	3 ^a	4,5 ^a	7,8 ^a	6 ^b
Fatty acids						
16:0	5.1	4.9	4.9	4.8	4.9	4.8
18:0	1.7	1.8	1.8	1.9	1.8	1.8
18:1	58.4	60.0	60.2	59.3	60.1	59.9
(<i>Z</i>)-18:2	21.1	20.5	20.4	21.5	20.4	20.8
(<i>E</i>)-18:2	n.d. ^c	n.d.	n.d.	n.d.	0.1	0.4
(<i>Z</i>)-18:3	9.65	8.54	8.44	8.4	8.55	7.24
(<i>E</i>)-18:3	0.05	0.06	0.06	n.d.	0.05	1.26

^aBleached oil, ^bDeodorized oil, ^cNot detected

Experiments at lower temperatures lasted longer in order to obtain similar level of isomerization in all tests. Because of their long duration, the tests were conducted in discontinuous way, except for the operations not longer than 6 hours (experiments 7 and 8) and experiment 3, which lasted 48 hours.

Degradation and geometrical isomerization of linoleic and linolenic acid was followed by analysis of fatty acid composition and (*E*) fatty acid content.

Table 7
Conditions of laboratory deodorizations

Experiment no.	Temperature °C	Time h	Stripping medium	Mode of operation
1	210	65	Nitrogen	Discontinuous
2	220	47	Steam	Discontinuous
3	230	48	Nitrogen	Continuous
4	235	12	Steam	Discontinuous
5	235	12	Nitrogen	Discontinuous
6	250	11	Steam	Discontinuous
7	250	6	Steam	Continuous
8	270	2	Steam	Continuous

4.1.1. Degradation of polyunsaturated fatty acids

Earlier studies about vegetable oils subjected to long-term heating mainly cover the users' point of view, e.g., heating in the presence of air and deep-frying of different foods. T. Kemény et al. (1992) reported 3.3-3.7% decrease in linoleic acid after four times 6-hour heating of refined sunflower oil at 180°C. Heating double fractionated palm olein at 180°C during 50 hours, Yoon et al (1987) found a 18.3% decrease in linoleic acid and a 62% decrease in linolenic acid content.

Heating linseed oil under nitrogen, De Greyt (1997) concluded that reactions (such as cyclization and conjugation) resulting in a decrease in linolenic acid content are only observed after heating >2 hours at 270°C.

In the present study, degradation of linoleic and linolenic acids during prolonged heating (deodorization) under vacuum was characterized. No noticeable change in the linoleic acid content was found under the conditions of laboratory experiments. The results are summarized in Attachment 2. The variations in the measured values were not significant even at a temperature as high as 270°C.

On the contrary, the total linolenic acid content decreased by 5.5 to 11.1% related to the initial values during the operations, and a linear relationship between the logarithm of the linolenic acid content and the duration of heating was observed (Attachment 3). The linolenic acid degradation is well described by the following equation:

$$(tot18:3)_t = (tot18:3)_{t=0} \cdot 10^{-k_{dLn} \cdot t}$$

or

$$\log (tot18:3)_t = -k_{dLn} \cdot t + \log (tot18:3)_{t=0} \quad \text{Equation 13}$$

where

$(tot18:3)_{t=0}$ is the initial total linolenic acid content,

$(tot18:3)_t$ is the total linolenic acid content at time t ,

t is the duration of heating (h) and

k_{dLn} is the degradation coefficient (h^{-1})

This linear relation indicates that linolenic acid degradation during heating is a first-order reaction. The degradation coefficients at the temperature of the experiment can be measured as the slope of the regression straight lines (Table 13).

The coefficient increases exponentially with the temperature according to the Arrhenius law. Equation 26 describes the linear relationship between the logarithm of the degradation coefficient and the reciprocal of the absolute temperature.

$$\log k_{dLn} = \frac{-k_t}{T} + b \quad \text{Equation 14}$$

where

k_t is the temperature coefficient (K) and

b is a constant value.

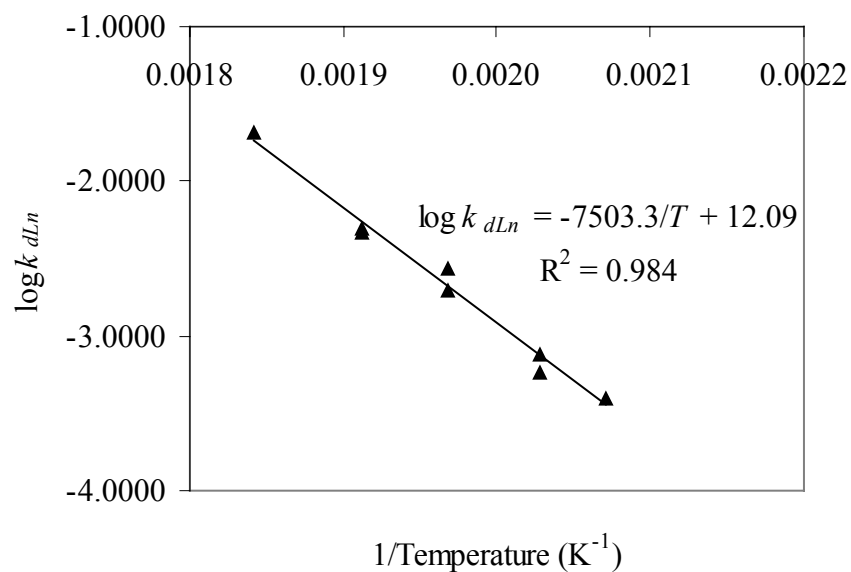
The parameters of the regression straight line (k_t and b) are determined in Figure 14.

Table 8
Measured degradation coefficients of linolenic acid

Experiment no.	Temperature °C	$k_{dLn} \cdot 10^4$ h^{-1}	y intercept	R^2
1	210	3.91	0.9883	0.958
2	220	5.78	0.9334	0.948
3	220	7.67	0.9289	1.000
4	235	19.9	0.9216	0.963
5	235	27.4	0.9229	0.978
6	250	47.1	0.9398	0.930
7	250	49.6	0.9344	0.958
8	270	203.7	0.9218	0.895

Figure 25

Effect of temperature on degradation coefficient of linolenic acid k_{dLn} .



Given k_t and b , the degradation coefficients of linolenic acid can be calculated for a wide range of temperature by Equation 27. (The calculated coefficients between 210 and 270°C are summarized in Table 14).

$$k_{dLn} = 12.09 \cdot 10^{-7503.3/T} \quad \text{Equation 15}$$

Example:

$$\text{At } 230^\circ\text{C } k_{dLn} = 12.09 \cdot 10^{-7503.3/(273+230)} = 14.9 \cdot 10^{-4} \text{ h}^{-1}$$

Table 9

Calculated degradation coefficients of linolenic acid

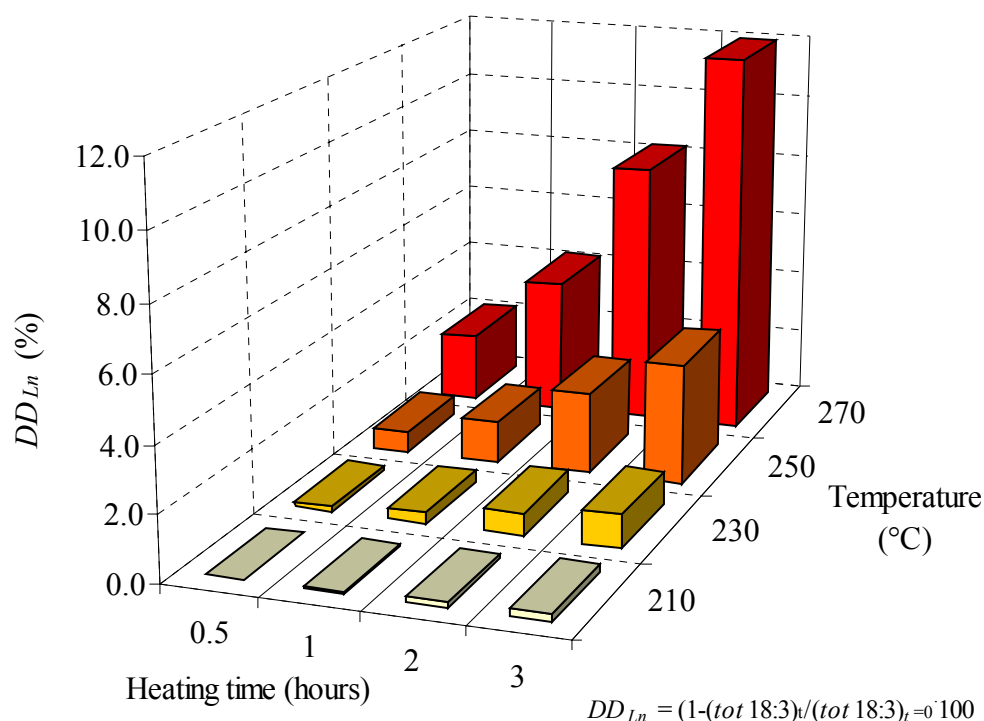
Temperature, °C	$k_{dLn} \cdot 10^4$ h ⁻¹
210	3.6
220	7.4
230	14.9
240	29.1
250	55.4
260	102.9
270	187.0

The linolenic acid loss during deodorization can be considered by means of Equation 15 and 13. In Figure 26 the degree of linolenic acid degradation (DD_{Ln}) is calculated for a temperature range from 210 to 270°C and times limited to 0.5-3 hours. DD_{Ln} is defined as the percentage of the decrease in total linolenic acid content). No remarkable loss can be noticed under regular deodorization conditions. Example: Deodorizing a rapeseed oil (initial linolenic acid content: 10%) at 230°C for 2 hours, the calculated total linolenic acid content at the end of the operation is 9.93%. This corresponds to a very low DD_{Ln} , 0.7%.

On the other side, exposing the oil to high temperature the linolenic acid degradation increases fast. At 270°C, 4% of the linolenic acid disappears during one

hour, after 3 hours the degree of degradation is 12%, which means a faster degradation than presumable from De Greyt's (1977) data.

Figure 15
Influence of deodorization conditions on the degree of linolenic acid degradation



4.1.2. Isomerization of polyunsaturated fatty acids

Great number of studies is available, in which formation of (*E*)-isomer fatty acid during deodorization is discussed. The degree of isomerization is determined by the temperature and the time of the operation (Devinat et al. 1980, Kellens 1997), no influence of steam or pressure can be observed (Jawad et al. 1983). According to Denecke (1995) and Wesdorp (1996) minor components like phosphorus and iron do not affect the reaction.

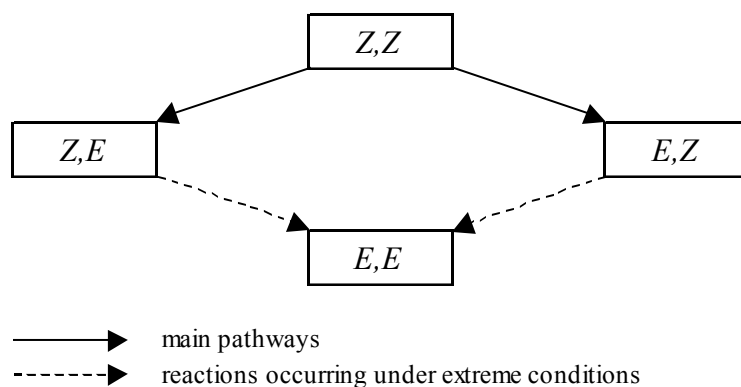
Measurements by O'Keefe et al. (1993) and Wolff (1993b) showed that the disappearance of linolenic acid follows a first order kinetic and the isomerization

rate constant varies with the temperature according to Arrhenius' law. Leon-Camacho et al. (2001) investigated the geometrical isomerization of linoleic acid. They concluded that under the circumstances of industrial deodorization the reaction can be considered zero ordered.

The rate of linolenic acid isomerization is 13-14 times higher than that of linoleic acid (Wolff 1992 and O'Keefe et al. 1994). Except these data on the relative rate of isomerization, no detailed study comparing the kinetics of geometrical isomerization of linoleic and linolenic acids has been published.

Concerning the isomerization of linoleic acid, primarily two geometrical isomers, (9*Z*,12*E*)- and (9*E*,12*Z*)-linoleic acid form (Figure 27). The proportion of the *Z,E* configuration was found slightly higher than that of the *E,Z* configuration. The (9*E*,12*E*)-linoleic acid isomer forms in a two-step reaction, but only at extreme conditions. Pudiel and Denecke (1997) reported that 300°C / 3 hours was necessary to obtain detectable quantity of this isomer.

Figure 16
Geometrical isomerization pathways of linolenic acid (Pudiel and Denecke 1997)

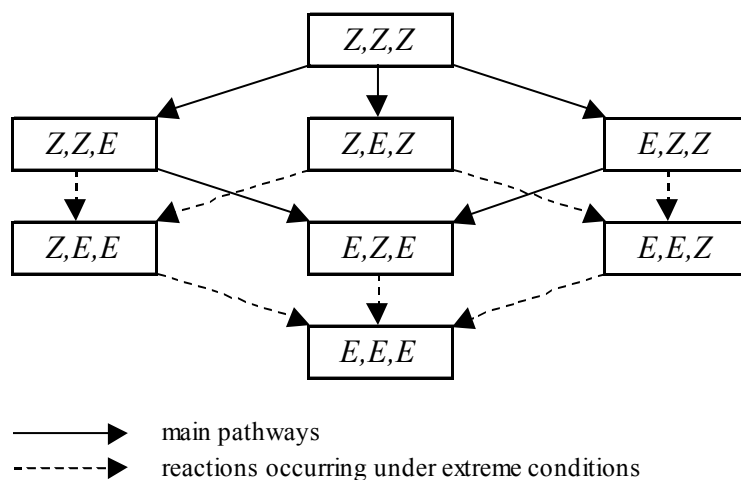


Considering the geometrical isomers of linolenic acid, generally four (*E*)-isomers can be detected in vegetable oils deodorized under regular conditions. Studies by the subsequent authors (O'Keefe 1994, Bertoli et al. 1996, Wolff 1997) showed that

the two main isomers are (9Z,12Z,15E)- and (9E,12Z,15Z)-linolenic acids, the two minor isomers are (9Z,12E,15Z)- and (9E,12Z,15ZE)-linolenic acids. In Figure 28 the alternative pathways of the reaction is summarized.

In the first step of isomerization mono-(*E*)-isomers appear. There is no simple relationship between the disappearance of the individual mono-(*E*)-isomers and the formation of the (9E,12Z,15E)-isomer. High deodorization temperature initiates the formation of the (9E,12Z,15E)- isomer via the (9Z,12Z,15E)- or (9E,12Z,15Z)-isomers in a second step of the reaction. The other possible isomers were not detected after 3-hour deodorization at 275°C (Pudel and Denecke 1997). After heating linseed oil in sealed ampoules at 245°C for 8 hours, Wolff (1993b) detected all the eight geometrical isomers of linolenic acid.

Figure 17
Geometrical isomerization pathways of linolenic acid (Pudel and Denecke 1997)

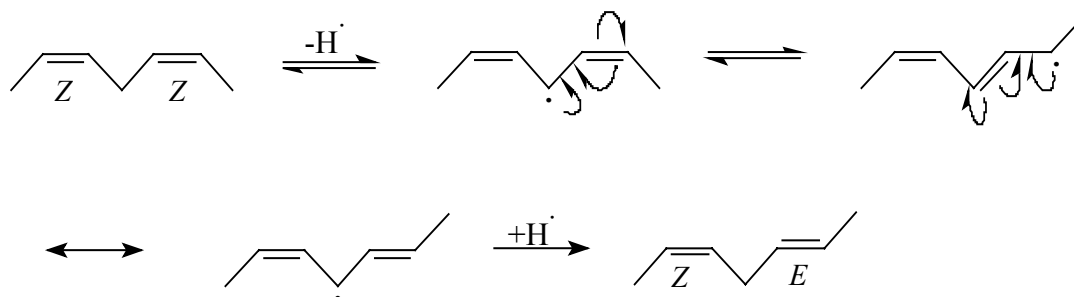


It is known that the distribution pattern of the geometrical isomers of linolenic acid varies in a very narrow range in commercial edible oils but only limited data is available about its dependence on the degree of isomerization (Wolff 1993, De Greyt 1997). To explain the isomerization of adjacent methylene-interrupted double bonds (Figure 29), Wolff et al. (1996) postulated the existence of a transient free

radical on the methylene group.

Figure 18

Geometrial isomerization of adjacent methylene-interrupted double bonds.
Reaction mechanism (Wolff et al. 1996)



Although it is still not clear, how this intermediate free radical is formed, but it should be stabilized by the five available electrons. The isomerization may then occur in both directions resulting in two mono-(*E*) isomers in roughly equal amounts. An important step in the reaction is the formation of an unstable conjugated *Z-Z* radical, that would give rise (after rotation of the former (*Z*)-ethylenic bond) to the more stable *E-Z* methylene-interrupted structure.

A *Z-E* methylene-interrupted structure is stabilized against further effect of heating, (*9Z,12Z,15E*)- and (*9E,12Z,15Z*)-isomers of α -linolenic acid can produce only (*9E,12Z,15E*)-isomer. Being unable to generate a new radical, the (*9Z,12E,15Z*)-linolenic acid can be considered as an end-product of the reaction. By other words, two adjacent methylene-interrupted double bonds can not both have the *E* configuration. This explains the experimental observations that the level of (*9E,12E*)-linoleic acid and the amount of (*9Z,12E,15E*)- and (*9E,12E,15Z*)-linolenic acid isomers is not detectable that even under drastic conditions such as 270°C, 2-2.25 hours (Wolff 1996, De Greyt 1997).

In the laboratory experiments of present study, 3.84 to 6.33% of total (*E*)-linolenic acid isomer content was achieved by the end of the operations. These values correspond to degrees of isomerization ranging from 49.3 to 79.0%. The degree of

isomerization of linoleic and linolenic acid (DI_L and DI_{Ln}) is defined as the percentage ratio between the total (E)-linoleic or -linolenic acid isomer content and the corresponding total linoleic or linolenic acid content. The total (E)-isomer content for linoleic acid ranged between 0.79 and 2.15%, representing DI_L of 3.7 to 10.4%. Results are summarized in Attachment 2.

Formation of geometrical isomers of polyunsaturated fatty acids during deodorization can be characterized by the remaining fraction of the (Z)-linoleic (marked as ZL_t) and (Z)-linolenic (ZLn_t) acid at time t . The (Z)-linoleic acid isomer fraction of a sample at time t is the ratio between its (Z)-linoleic acid isomer content ($Z18:2$) and its total linoleic acid content ($tot18:2$). Total linoleic acid content means the sum of the (Z)- and (E)-isomer content. In the same way, the (Z)-linolenic acid isomer fraction of this sample is the ratio between its (Z)-linolenic acid isomer content ($Z18:3$) and its total linolenic acid content ($tot18:3$). Total linolenic acid content means the sum of the (Z) and (E)-linolenic acid isomer content.

Linoleic acid (Equation 28):

$$(Z18:2)_t = (tot18:2)_t \cdot 10^{-K_{il,t} \cdot t}$$

$$ZL_t = 10^{-K_{il,t} \cdot t}$$

Linolenic acid (Equation 29)

$$(Z18:3)_t = (tot18:3)_t \cdot 10^{-K_{iln,t} \cdot t}$$

$$ZLn_t = 10^{-K_{iln,t} \cdot t}$$

Values at time 0 correspond to the initial oil (before heating), except for experiment 8, in which the first sample was taken just when the operating temperature of 270°C was reached. For this latter sample, the fact that ZL_t and ZLn_t are less than 1 proves that isomerization has already occurred during the heating up period. This may also

occur at lower temperatures but to a much lesser extent. In case of experiment 6 a fully refined oil was re-deodorized, which explains that the initial values of ZL_t and ZLn_t are less than 1.

Plotting the logarithm of ZL_t and ZLn_t fractions as a function of the deodorization time, strong linear correlation can be noticed for each trial (Attachment 4-5). This linear relationship confirms that geometrical isomerization of linoleic and linolenic acid is a first-order reaction. Table 15 summarizes the characteristics of the regression straight lines.

Table 10
Measured isomerization coefficients of linoleic and linolenic acid

Exp. no.	Temperature °C	$K_{iL} \cdot 10^4$ h ⁻¹	y-intercept	R ²	$K_{iLn} \cdot 10^4$ h ⁻¹	y-intercept	R ²
1	210	2.64	0.0003	0.993	55.3	-0.0059	0.999
2	220	4.53	0.0012	0.986	92.3	-0.0050	0.999
3	220	4.50	0.0000	1.000	93.4	-0.0030	1.000
4	235	14.0	0.0004	0.996	251.4	-0.0079	0.999
5	235	15.3	0.0002	0.994	246.6	-0.0003	0.999
7	250	41.2	-0.0030	0.998	557.6	-0.0927	0.993
6	250	43.2	0.0007	0.991	631.3	-0.0193	0.979
8	270	150.1	-0.0019	0.991	1887.0	-0.0423	0.996

The absolute values of the slopes measure the rate constants of the isomerization reaction of linoleic and linolenic acids, K_{iL} and K_{iLn} respectively. The y-intercepts slightly differ from zero, which may partly be attributed to the uncertainty of the measurements, but more likely to the fact that during the heating up phase isomerization already occurred in a small extent. This is confirmed by the observation that for linolenic acid (more prone to isomerization) the y-intercept was always negative, but in case of linoleic acid was negative values could only be found at higher temperatures. In experiment 6 the use of a deodorized oil had no

significant effect on the rate constant, it only resulted in an increase of the y-intercept.

As it is demonstrated in Figure 30 and 31, the measured rate constants of isomerization followed the Arrhenius' law both for linoleic and linolenic acids.

Definite linear relationship was noticed between the measured isomerization constants and the reciprocal of the absolute temperature in both cases. Thus, the isomerization constants can be calculated by using Equation 18 and 19.

Figure 19

Effect of temperature on isomerization coefficient of linoleic acid K_{iL}

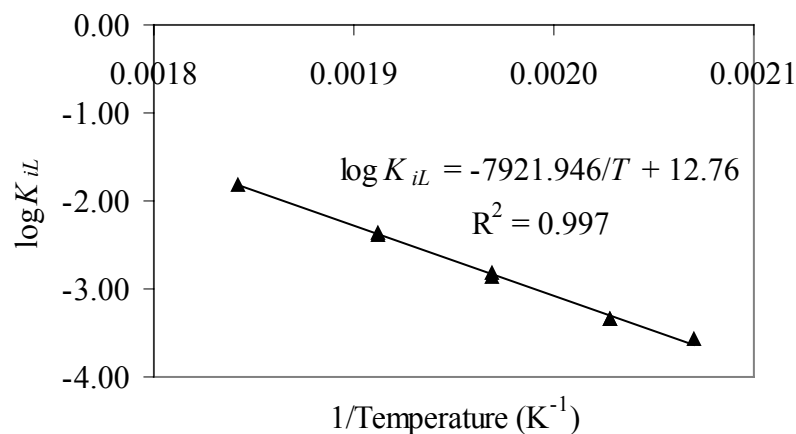
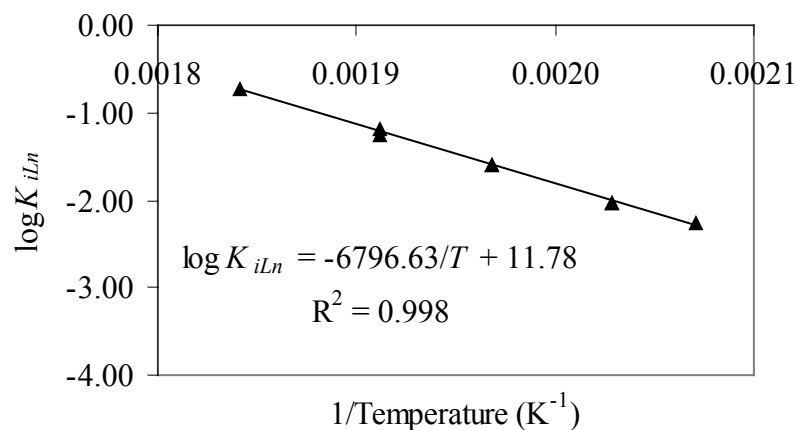


Figure 20

Effect of temperature on isomerization coefficient of linolenic acid K_{iLn}



$$K_{iL} = 12.76 \cdot 10^{-7921.95/T} \quad \text{Equation 18}$$

$$K_{iLn} = 11.78 \cdot 10^{-6796.63/T} \quad \text{Equation 19}$$

The calculated isomerization constants for the temperature range of 190-270°C are listed in Table 16. The obtained K_{iLn} values are similar, slightly lower than those of Wolff (1993b) obtained when heating linseed oil under vacuum. The calculations confirm that the isomerization of linolenic acid is much faster than that of linoleic acid. Both K_{iL} and K_{iLn} doubles approximately by a 10°C increase in temperature.

Table 11
Calculated isomerization coefficients of linoleic and linolenic acid

Temperature °C	This study		Wolff (1993b)
	$K_{iL} \cdot 10^4$ h ⁻¹	$K_{iLn} \cdot 10^4$ h ⁻¹	$K_{iLn} \cdot 10^4$ h ⁻¹
190	0.4	12.6	9.3
210	2.3	51.1	
220	4.9	98.6	72.4
230	10.2	185.3	
240	20.8	339.8	
250	41.0	608.9	
260	78.9	1067.5	927.0
270	148.2	1833.1	

4.2. VALIDATION OF THE MODEL BY PILOT PLANT SCALE EXPERIMENTS

Pilot plant scale deodorization tests were performed to check the reliability of the model established at laboratory scale. It was checked by statistical calculations if there was any significant difference between the results obtained at pilot plant scale

and those of calculated from the laboratory model.

Degummed bleached rapeseed oil used in these experiments contained 21.3% linoleic and 8.3% linolenic acid. No (*E*)-isomers of linoleic and linolenic acids were found in the raw material (Table 17).

Pilot plant scale tests were carried out according to the procedure described in chapter 3.1.2. Three pilot plant tests were carried out under the circumstances listed in Table 18. The operations lasted 24, 48 and 82 hours at temperatures of 230, 220 and 210°C respectively. Similarly to the laboratory experiments, degradation and geometrical isomerization of linoleic and linolenic acid were followed by analysis of fatty acid composition and (*E*) fatty acid content.

Table 12
Fatty acid composition (%) of bleached
rapeseed oil used in pilot plant experiments

Oil id.	G
Experiment no.	9-11
Fatty acids	
16:0	4.8
18:0	1.8
18:1	60.1
(<i>Z</i>)-18:2	21.3
(<i>E</i>)-18:2	n.d. ^a
(<i>Z</i>)-18:3	8.3
(<i>E</i>)-18:3	n.d.

^aNot detected

Table 13
Conditions of pilot plant scale deodorizations

Experiment no.	Temperature °C	Time h	Stripping medium	Mode of operation
9	210	86	Nitrogen	Continuous
10	220	48	Nitrogen	Continuous
11	230	24	Nitrogen	Continuous

4.2.1. Degradation of polyunsaturated fatty acids

No noticeable change in the total linoleic acid content was noticed in the pilot plant tests, which confirms the observation made during the laboratory experiments. A characteristic decrease of linolenic acid amount was found under the conditions of pilot plant experiments (Attachment 6). The linolenic acid content decreased from 8.3 to 7.7-7.8%, which means a 6-7% loss during extremely long heating.

The reliability of the laboratory model was checked by two statistical calculations. The differences between the values of total linoleic acid content measured from the pilot plant experiments and those calculated using the theoretical model were analyzed. The Student's t-test and the Abbe test were conducted on samples heated at each temperature for both linoleic and linolenic acid (Table 19).

Performing the Student's t-test, the absolute value of the test statistic ($|t_{dLn}|$) was lower than the critical $t_{0.05}$ value in each case. In other words the mean of the difference between the measured and the theoretical values of total linolenic acid content is zero with 95% confidence at 210, 220 and 230°C equally.

The Abbe test showed that no systematic order of the differences can be found at a confidence level of 95% in all cases, as the values of the test statistic (R_{dLn}) were greater than the critical $R_{0.05}$ values.

Table 14
Statistical analysis of the differences between the theoretical and measured linolenic acid content

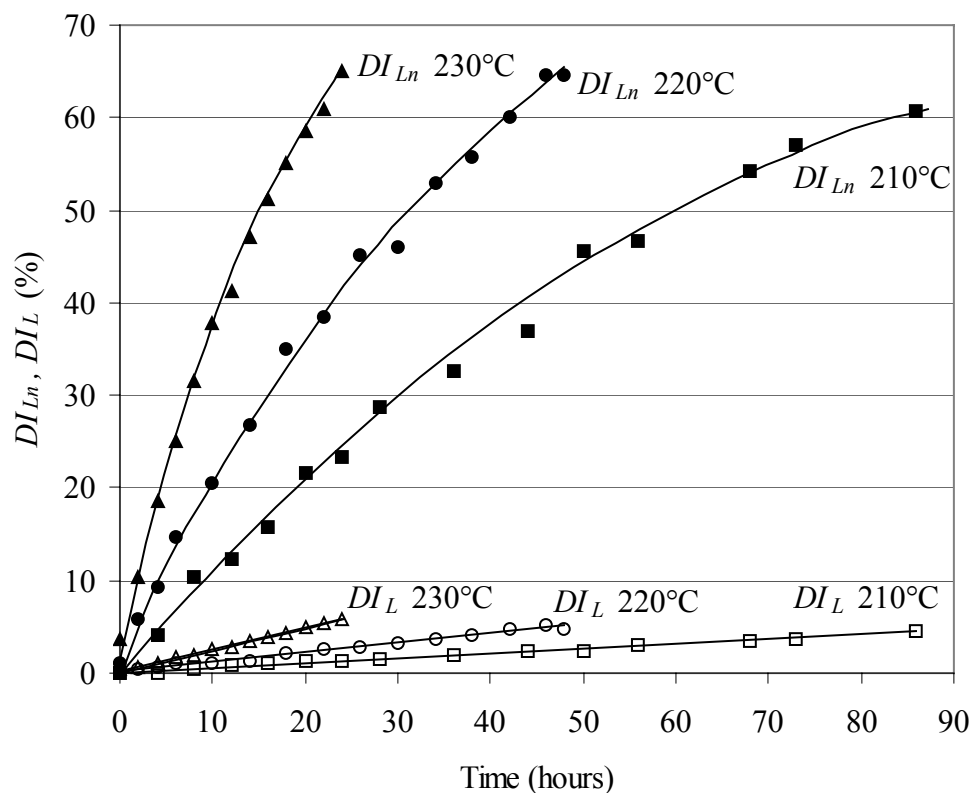
Temperature °C	Student's t-test		Abbe test	
	$ t_{dLn} $	$t_{0.05}$	R_{dLn}	$R_{0.05}$
210	0.93	2.13	0.71	0.64
220	2.08	2.12	0.74	0.61
230	0.99	2.16	0.86	0.61

Therefore, the model provides accurate information on the linolenic acid degradation.

4.2.2. Isomerization of polyunsaturated fatty acids

Concerning geometrical isomerization, 0.95, 1.01 and 1.23% of total (*E*)-linoleic acid isomers were reached by the end of the pilot plant operation at 210°C/86h, 220°C/48h, and 230°C/24h (Attachment 6). These values correspond to isomerization degrees of 4.5, 4.7 and 5.8%, respectively (Figure 32). The total (*E*)-isomer content for linolenic acid was 4.62, 5.04 and 5.02% at 210, 220 and 230°C, representing DI_L 's of 60.8, 64.5 and 65.0% respectively.

Figure 21
Degree of isomerization of linolenic and linolenic acid as a function of heating time.



Under the pilot plant conditions, the (*E*) fatty acid content was found to increase slightly during the heating up period. (Values at time 0 correspond to the oil at

moment of reaching the operating temperature.) Also, the higher the temperature to be reached the higher the amount of (*E*) fatty acids formed. No (*E*)-isomers were detected during heating to 210°C, but 0.1% (*E*)-linoleic and 0.5% (*E*)-linolenic acid isomers were found when heating to at 230°C.

In order to eliminate this effect, only (*E*)-isomers formed after reaching the operating temperature were taken into consideration. To characterize the development of (*E*)-linolenic acid formed strictly at the operating temperature, the (*E*)-linolenic acid content measured at time t was transformed as follows:

$$(E18:3)_t' = (E18:3)_t - (E18:3)_{t=0} \quad \text{Equation 32}$$

where

$(E18:3)_t'$ is the (*E*)-linolenic acid formed after reaching the operating temperature,

$(E18:3)_t$ is the (*E*)-linolenic acid content measured at time t and

$(E18:3)_{t=0}$ is the amount of *E* linolenic acid at time $t=0$.

In the case when no measurable (*E*)-isomer forms during heating up, $(E18:3)_t' = (E18:3)_t$. The values of *E* linoleic acid content measured at time t $(E18:2)_t$ were transformed similarly.

The consequence of the isomerization during the warm up phase was that by the time the operating temperature was reached ($t=0$), the concentration of (*Z*)-linoleic and -linolenic acid had begun to decrease. This difference can be compensated for using a modified fatty acid composition in theoretical calculations:

$$(Z18:3)_{t=0,theo} = (Z18:3)_i - (E18:3)_{t=0} \quad \text{Equation 33 and}$$

$$(E18:3)_{t=0,theo} = 0 \quad \text{Equation 34, thus}$$

$$(tot18:3)_{t=0,theo} = (Z18:3)_i - (E18:3)_{t=0} \quad \text{Equation 35}$$

(and similarly for linoleic acid) where

$(Z18:3)_i$ is the measured initial (Z)-linolenic acid content before deodorization,

$(Z18:3)_{t=0}$ is the measured (Z)-linolenic acid content at time $t=0$,

$(Z18:3)_{t=0,theo}$ is the theoretical (Z)-linolenic acid content at time $t=0$ and

$(tot18:3)_{t=0,theo}$ is the theoretical total linolenic acid content at time $t=0$.

Statistical evaluation of the pilot plant data and the theoretical values was performed taking into account the above considerations (Table 20). Concerning the Student's t-test, the absolute values of the test statistic ($|t_L|$ and $|t_{Ln}|$) were lower than the critical $t_{0.05}$ values in each case. This suggests that in case of linoleic and linolenic acid the difference between the measured and the theoretical *E* fatty acid content is not significant.

The Abbe test proved the absence of systematic order of the differences at a confidence level of 95% in all cases, as the values of the test statistic (R_L and R_{Ln}) were greater than the critical $R_{0.05}$ values at 210, 220 and 230°C equally.

Table 15

Statistical analysis of the theoretical and measured trans linoleic and linolenic acid content

Temperature °C	Student t-test			Abbe test		
	$t_{0.05}$	$ t_L $	$ t_{Ln} $	$R_{0.05}$	R_L	R_{Ln}
210	2.13	0.80	2.08	0.64	0.70	1.12
220	2.12	1.06	1.30	0.61	0.63	0.62
230	2.16	1.18	0.04	0.61	0.99	0.73

We can conclude that the model provides reliable information on the formation of (*E*)-linoleic and -linolenic acid isomers.

4.2.3. Distribution of the individual geometrical fatty acid isomers

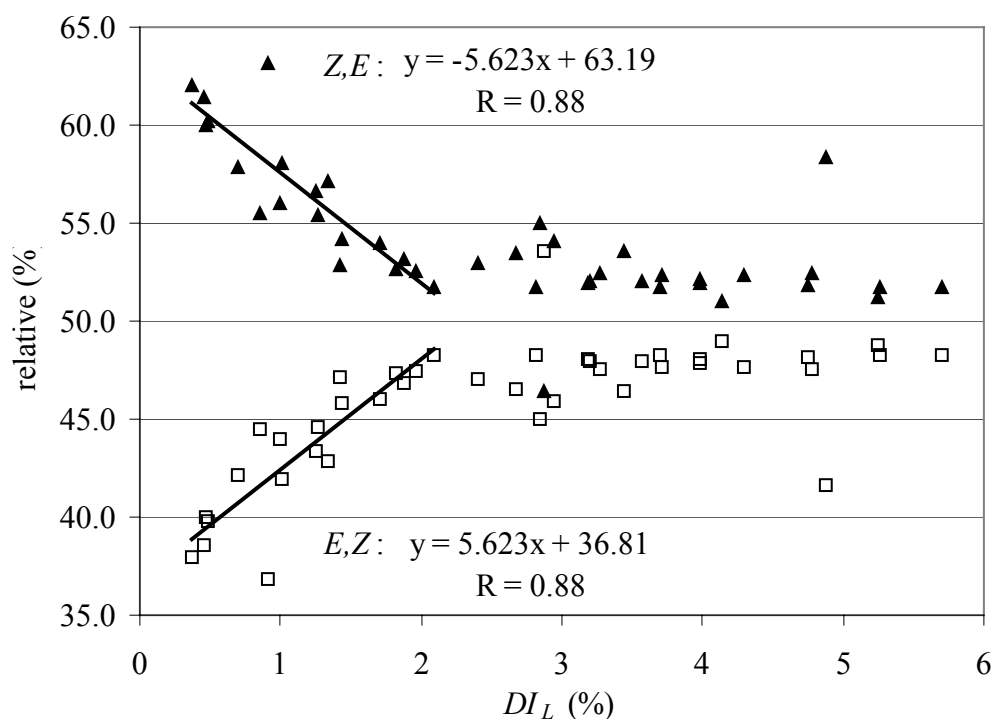
Linoleic acid

During the experiments two geometrical isomers, (9*Z*,12*E*)- and (9*E*,12*Z*)-linoleic acid appeared, hereafter referred as *Z,E* and *E,Z* linoleic acid.

No (9*E*,12*E*)-isomer was detected under the pilot plant conditions. In Figure 33 the relative amounts of *Z,E*- and *E,Z*-linoleic acids are plotted as a function of DI_L . The percentage of the *Z,E* isomer was higher in all cases except one sample. With increasing DI_L the amount of these two compounds get closer to each other. At $DI_L \geq 4\%$ the relative amount of the *Z,E* and *E,Z* isomer was 51-52 and 49-48%. For DI_L 's lower than 2.1, linear relationship was observed between the relative amount of the two geometrical isomers and the degree of isomerization.

Figure 22

Relative amounts of *Z,E*- and *E,Z*-linoleic acid isomers as a function of the degree of isomerization ($DI_L = (E18:2)/(tot18:2); 100$)



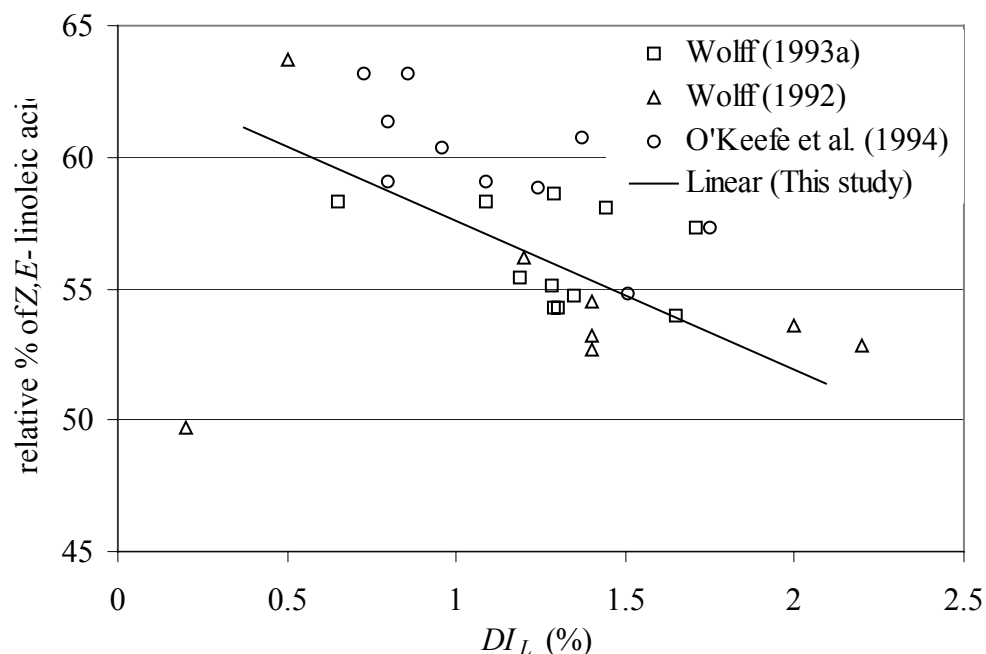
Calculated as the intercepts of the regression straight lines, the initial probabilities of the formation of *Z,E*- and *E,Z*-linoleic acids are 63.2 and 36.8% respectively. (Data at $DI_L < 0.4$ were not taken into consideration because at such low values the

relative amount of the individual isomers is not reliable.) There is no other data available on the initial probabilities of these isomers.

Figure 34 indicates that the relative amount of *Z,E* linoleic acid and the corresponding DI_L values determined in commercial refined oils by Wolff (1992 and 1993) correspond closely to the regression straight lines described above. The percentages of *Z,E* linoleic acid, calculated from the data of O'Keefe et al. (1994), are in similar range.

Figure 23

Relative amounts of Z,E- and E,Z-linoleic acid isomers in commercial refined oils.

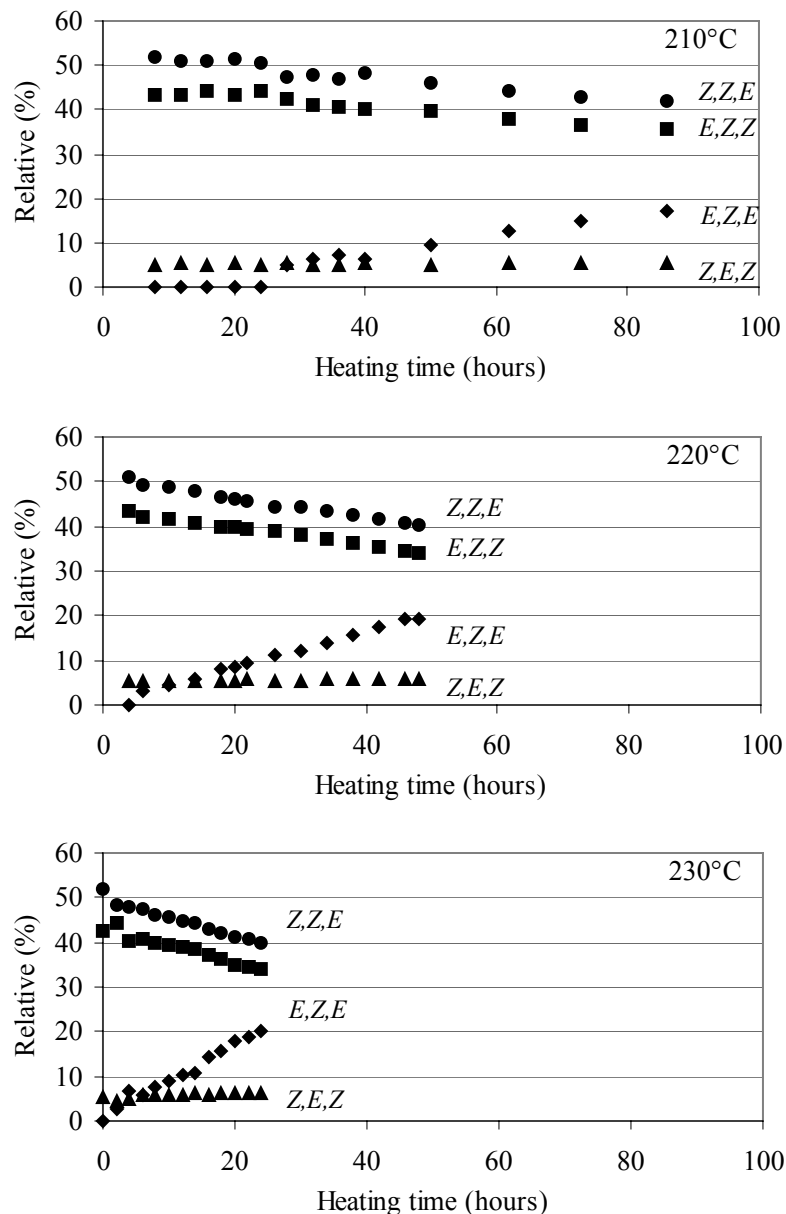


Linolenic acid

Four geometrical isomers of linolenic acid formed during heating as described in the literature. The (9*Z*,12*Z*,15*E*)-, (9*E*,12*Z*,15*Z*)-, (9*Z*,12*E*,15*Z*)- and (9*E*,12*Z*,15*E*)- isomers will be referred hereafter as *Z,Z,E*-, *E,E,Z*-, *Z,E,Z*- and *E,Z,E*-linolenic acid. During the pilot plant experiments of this study, the relative amount of individual geometrical isomers of linolenic acid developed as demonstrated in Figure 35.

Figure 24

Influence of the deodorization conditions on the relative amount of *E*-linolenic acid isomers

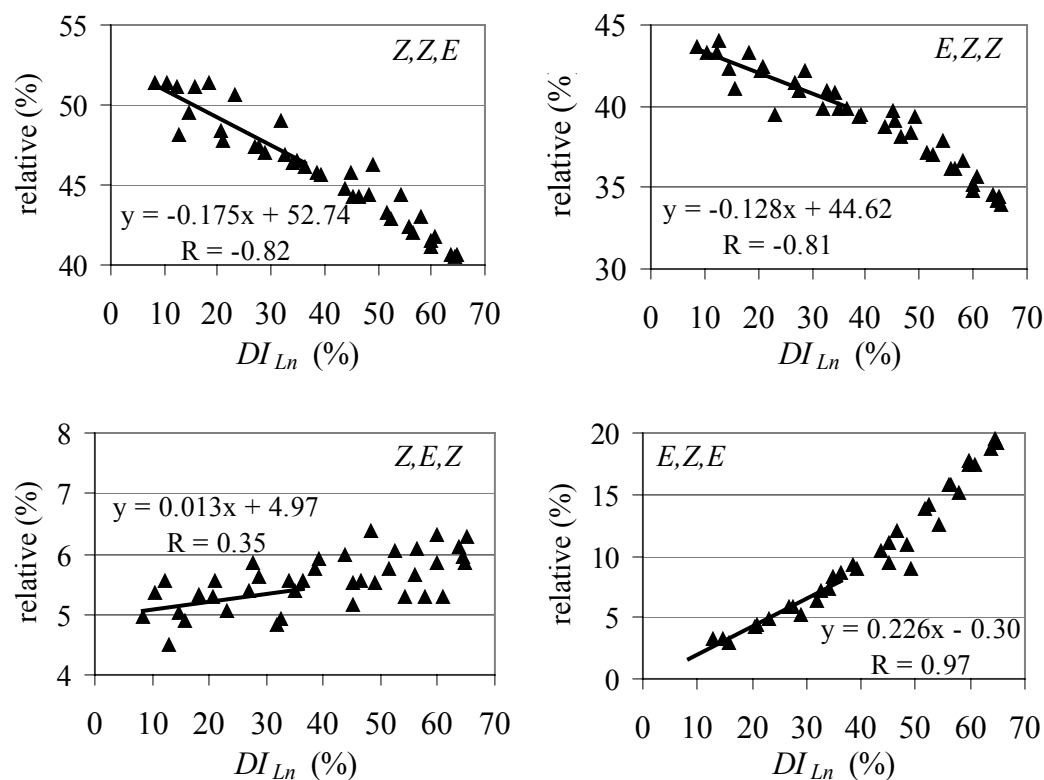


Under mild conditions only the two main isomers (*Z,Z,E* and *E,Z,Z*) was quantified in small amounts. The *Z,E,Z* isomer reached a detectable quantity (0.03-0.05%) after 8 hours at 210°C, after 4 hours at 220°C or by the end of the heating up period in case of the experiment at 230°C. The detected amount represented 5-6% of the

total *E* linolenic acid content. This value remained unchanged during all experiments confirming that the *Z,E,Z* isomer can be considered as a kind of end product of the geometrical isomerization of linolenic acid. The *E,Z,E* isomer appeared after 28 hours at 210°C, after 6 hours at 220°C and already after 2 hours at 230°C. Until this point only mono-(*E*)-isomers were present and their relative percentage was constant. Once the *Z,E,Z* isomer appeared, its proportion increased linearly with time at the expense of the *E,E,Z* and *Z,E,E* isomers. This confirms that the formation of the *E,Z,E*-linolenic acid isomer follows a two-step reaction. To determine the relative amounts of geometrical isomers in the early stage of the reaction the distribution of the individual isomers is plotted as a function of DI_{Ln} .

Figure 25

Distribution of the (*E*)-linolenic acid isomers as a function of DI_{Ln}



In Figure 36 the data of the three pilot plant tests are combined (data at $DI_{Ln} < 6$

were not taken into consideration). A linear relationship was found for each individual isomer in a range limited to DI_{Ln} 's lower than 35%. In case of the Z,Z,E , E,Z,Z and E,Z,E isomers strong correlation was observed. The relative amount of the Z,Z,E isomer decreased slightly faster than that of the E,Z,Z component.

The adjusted values of the intercepts indicate that at the beginning of the reaction the probability of formation of individual geometrical isomers are 51.5, 43.6, 4.9 and 0% for the Z,Z,E , E,Z,Z , Z,E,Z and E,Z,E isomers respectively. (The intercept of E,Z,E isomer was considered 0, the others were adjusted accordingly.) As it can be seen in Table 21, the values obtained in this way correspond closely with the pattern described by Wolff (1993a and 1993b) and De Greyt (1997).

Table 16

Initial probability of formation of geometrical linolenic acid isomers (%).

Isomer	This study ^a	References		
		De Greyt(1997) ^b	Wolff(1993b) ^c	Wolff(1993a) ^d
Z,Z,E	51.5	51.2	53.3	52.1
E,Z,Z	43.6	44.9	41.9	41.7
Z,E,Z	4.9	5.7	4.7	6.2
E,Z,E	0	0	0	0

^aCalculated from pilot plant scale heating of rapeseed oil

^bCalculated from pilot plant scale heating of linseed oil

^cCalculated from laboratory scale heating of linseed oil

^dCalculated from the distribution of the geometrical isomers in commercial refined oils

4.3. RESEARCH AND INDUSTRIAL APPLICATION OF THE MODEL

In the field of research, the model was applied to calculate model the deodorization conditions and to produce a specially isomerized oil for external research partners for a nutritional project. In frame of the European Union project entitled "Nutritional and Health Impact of *E* Polyunsaturated Fatty Acids in European

Populations” the metabolic effect of (*E*)-linolenic acid isomers was investigated at different research institutes in Europe. Our role in the project was to provide an oil for the above studies with an extreme composition of (*E*)-isomer fatty acids. Fully refined oil containing 5% of (*E*)-linolenic acid and the possible lowest quantity of (*E*)-linoleic acid (max. 0.5%) was requested for the study. The term “selective isomerization” is used because the targeted ratio r between the (*E*)-linolenic acid and the (*E*)-linoleic acid content was very high ($r = 10$). A reference oil (“zero *trans*” oil) containing the possible lowest quantity of any *E* fatty acids was also demanded. Apart from the *E* fatty acid content, the other quality parameters had to meet fully refined oil specifications since the oils were used in a human diet.

Concerning the industrial practice, deodorization conditions were outlined, applying which the (*E*) fatty acid content of refined oils meet the current consumers’ demand. Secondly, industrial deodorizers were surveyed from the point of view of (*E*)-isomer formation, comparing the (*E*) fatty acid content of refined oils with the corresponding theoretical values.

4.3.1. General considerations

Given the kinetic model, some general observations can be made with particular attention to (i) the (*E*) fatty acid isomer content of deodorized oils (ii) the degree and selectivity of geometrical isomerization of polyunsaturated fatty acids and (iii) the time of deodorization.

(E) fatty acid isomer content

The (*E*)-linoleic acid isomer content of a deodorized oil can be expressed from equation 16 by adding $(E18:2)_t$ to its both sides:

$$(E18:2)_t = (tot\ 18:2)_t \cdot (1 - 10^{-K_L \cdot t}) \quad \text{Equation 24}$$

Similarly for linolenic acid from equation 17:

$$(E18:3)_t = (tot\ 18:3)_t \cdot (1 - 10^{-K_{Ln} \cdot t})$$

$$(E18:3)_t = (tot\ 18:3)_{t=0} \cdot 10^{-k_{dLn} \cdot t} \cdot (1 - 10^{-K_{Ln} \cdot t}) \quad \text{Equation 25}$$

Known the initial fatty acid composition of an oil, the above equations enable us to calculate the (*E*)-linoleic and (*E*)-linolenic content forming under different deodorization conditions. Additionally, it can be noticed that the (*E*)-linoleic acid content is practically not affected by the degradation, the total linoleic acid content remains unchanged during deodorization ($(tot18:2)_t = (tot18:2)_{t=0}$). Equation 25, on the other hand, takes into account the influence of linolenic acid degradation on the (*E*)-linolenic acid content.

Degree of isomerization

Transforming equations 16 and 17 according to the definition of the degree of isomerization, DI_L and DI_{Ln} can be calculated in the following way:

Linoleic acid:

$$DI_L = 100 \cdot (1 - 10^{-K_L \cdot t}) \quad \text{Equation 26}$$

Linolenic acid:

$$DI_{Ln} = 100 \cdot (1 - 10^{-K_{Ln} \cdot t}) \quad \text{Equation 27}$$

Since the reactions studied are of first order, the degree of isomerization of linoleic and linolenic acids is independent of the initial content of these fatty acids. DI_L and DI_{Ln} depend only on the deodorization temperature and time.

Selectivity of the isomerization

The selectivity S of the isomerization of linolenic acid in comparison with linoleic acid is defined by the ratio between the isomerization constants of these two fatty acids (K_{Ln}/K_L). Using the equations 16 and 17 the selectivity can be expressed as follows:

$$S = 10^{1125/T-0.98} \quad \text{Equation 40}$$

Contrary to the degree of isomerization, the selectivity only depends on the temperature of the operation. Increasing the temperature from 210°C to 270°C, its value decreases about by half (Table 22). In order to produce oil with 5% (*E*)-linolenic acid with maximum 0.5% (*E*)-linoleic acid, a high selectivity, therefore a low temperature is required.

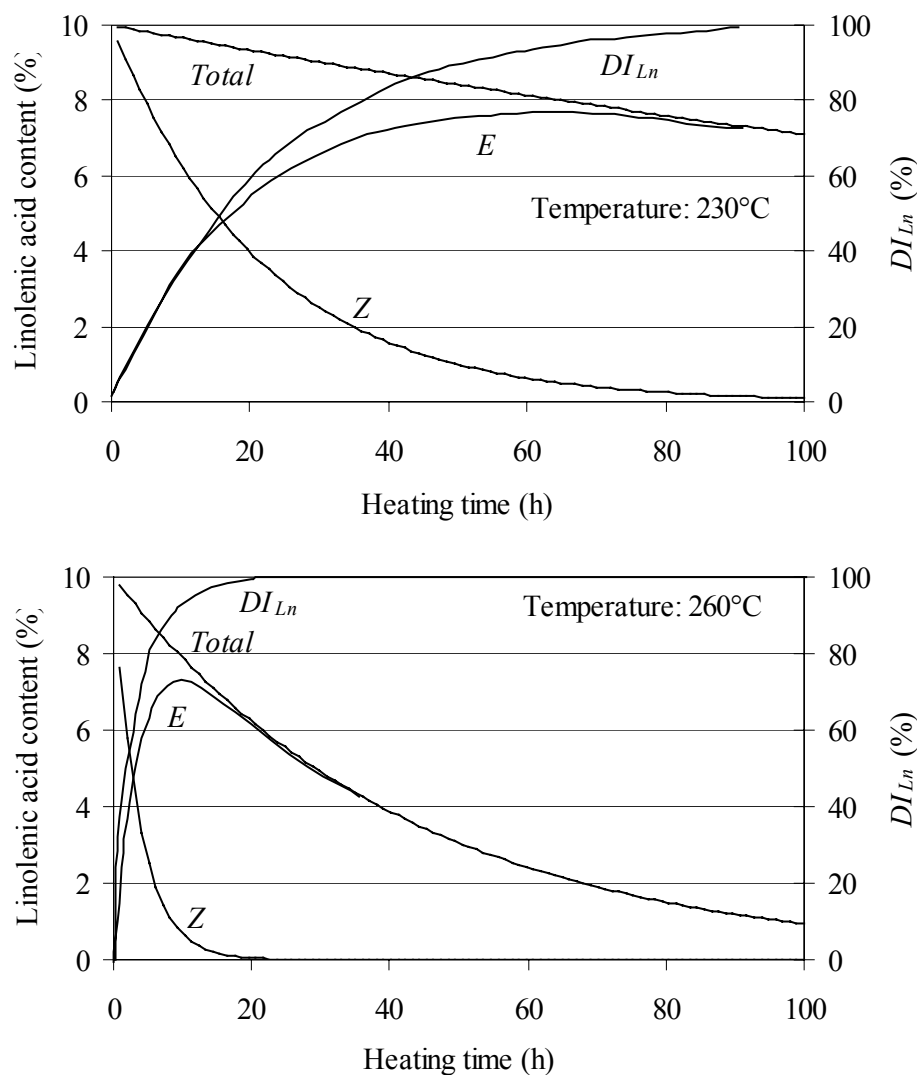
Table 17
Selectivity S of geometrical isomerization of linolenic vs. linoleic acid

Temperature, °C	S	Temperature, °C	S
210	22.3	250	14.8
220	20.0	260	13.5
230	18.1	270	12.4
240	16.3		

Influence of linolenic acid degradation on the (E)-linolenic acid content

At the temperature of deodorization, there is a competition between the isomerization and degradation of linolenic acid. In Figure 37 theoretical calculations are shown for rapeseed oil at moderate and high temperature (230 and 260°C). The figure suggests that total linolenic acid content decreases during heating. Consequently, the maximum (*E*)-isomer content that can be obtained during deodorization of a given oil is lower than the initial total linolenic acid content.

Figure 26
Effect of linolenic acid loss on geometrical isomerization of rapeseed oil.



The time t_{max} necessary to obtain the maximum value can be calculated from the first derivative with respect to t of equation 25. With this consideration:

$$t_{max} = -\frac{1}{K_{Ln}} \cdot \log\left(\frac{k_{dLn}}{k_{dLn} + K_{Ln}}\right) \quad \text{Equation 30}$$

This t_{max} reaction time depend only on the applied temperature and it is independent from the initial total linolenic acid content of the oil (Table 23). It decreases

approximately by half for each 10°C increase in temperature. The maximum (*E*)-isomer content varies evidently with the initial linolenic acid content, but only slight influence of the temperature can be observed.

Table 18

*Heating time (t_{max}) needed to reach the maximum (*E*)-linolenic acid content*

Temperature (°C)	t_{max} (h)	Temperature (°C)	t_{max} (h)
210	268	250	20
220	134	260	11
230	69	270	6
240	36		

Deodorization time

In order to calculate the time t , during which a given amount of (*E*)-linolenic acid isomers is formed at a certain temperature, Equation 25 has to be transformed:

$$t = \frac{1}{k_{dLn} + K_{Ln}} \cdot \log\left(\frac{(tot18:3)_{t=0}}{(tot18:3)_{t=0} \cdot 10^{-k_{dLn} \cdot t} - (E18:3)_t}\right) \quad \text{Equation 31}$$

This equation can be resolved by applying an iterative procedure until the value of t calculated in the left-hand side of the equation is equal to the value of t supposed in the right-hand side. In those cases when the degradation is negligible (the degradation coefficient approaches zero), the equation simplifies and it t can be determined without iteration. Concerning linoleic acid this simplification is fully justified:

$$t = \frac{1}{K_{Ln}} \cdot \log\left(\frac{(tot18:2)_{t=0}}{(tot18:2)_{t=0} - (E18:2)_t}\right) \quad \text{Equation 32}$$

4.3.2. Production of selectively isomerized oil for a nutritional study

The targeted (*E*)-isomer pattern of the selectively isomerized oil has been described

at the beginning of chapter 4.3. The high (*E*)-linolenic acid isomer content but especially the high ratio of the (*E*)-linolenic and (*E*)-linoleic acid isomers are quite different from the values characteristic of commercially available refined vegetable oils. In order to obtain an oil containing 5% (*E*)-linolenic acid and 0.5% (*E*)-linoleic acid, attention has to be paid to the selection of the raw material, and the deodorization conditions for the selected oil has to be thoroughly chosen by using the model.

4.3.2.1. Raw material selection, calculation of deodorization conditions

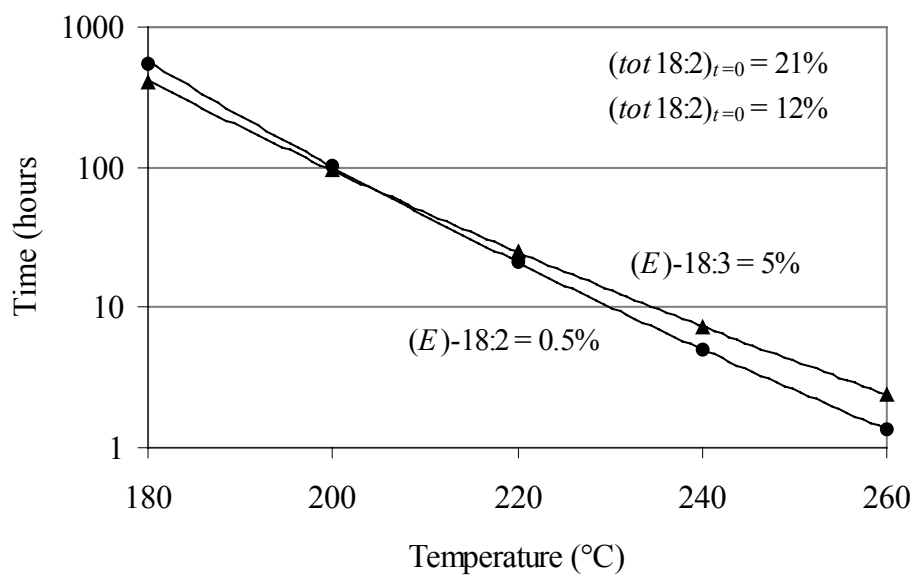
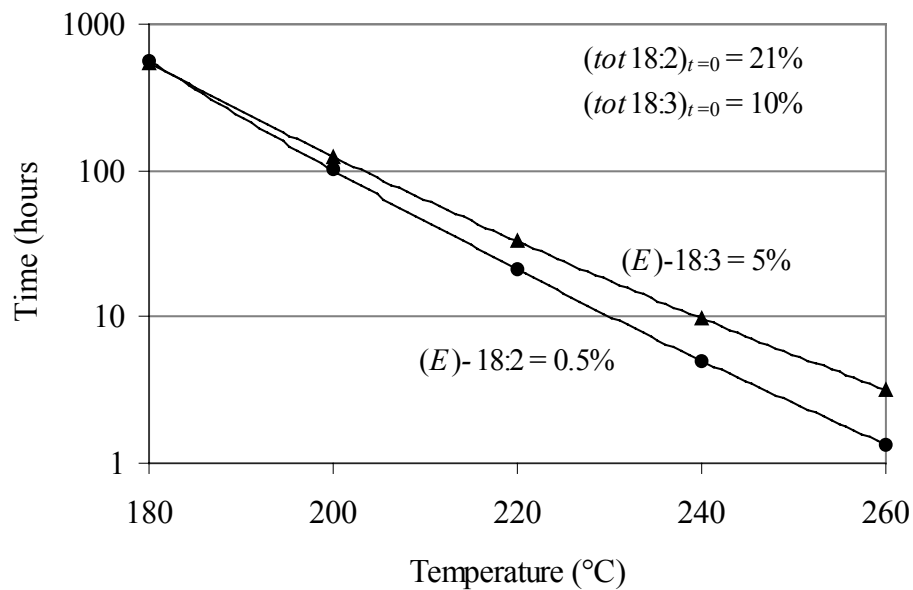
It is evident that the fatty acid composition of the raw material has a strong influence on the r ratio of (*E*)-linolenic and (*E*)-linoleic acid. Principally, oils rich in linolenic acid but low in linoleic acid are of interest. From this point of view, the fatty acid pattern of rapeseed oil is the most favourable. Despite of containing significant amount of linolenic acid, soybean and walnut oils are not suitable because of their high linoleic acid content.

As far as the process conditions are concerned, the (*E*)-linolenic acid content can be easily raised to 5% simply by applying high temperature. Conversely, to keep the (*E*)-linoleic acid content at maximum 0.5% in order to achieve the targeted r value of 10 makes difficulties.

On Figure 38 the time necessary to obtain 5% (*E*)-linolenic and 0.5% (*E*)-linoleic acid is plotted in function of temperature. At regular deodorization temperatures 0.5% (*E*)-linoleic acid forms much faster, during 5 hours at 240°C, while to achieve 5% (*E*)-linolenic acid 10 hours are necessary under the same conditions. In other words the r ratio is far from the desired 10. Improving the selectivity by applying lower temperature, the two lines get closer and cross ($r=10$) at 185°C in case of the initial fatty acid composition given in the figure. On the other hand, such a low temperature would require an excessively long deodorization time, which is not feasible even on a pilot plant scale.

Figure 27

Time needed to obtain 5% (E)-18:3 and 0.5% (E)-18:2. Influence of initial 18:3 content



To shift the intersection point towards higher temperatures (shorter times), the only possibility is to select an oil containing more linolenic acid but not richer in linoleic acid. As it is demonstrated in Table 24, an initial linolenic acid content of 12% is

necessary to decrease the deodorization time to about 70 hours.

Table 19

Deodorization conditions to obtain 5% (E)-18:3 and 0.5% (E)-18:2 content:
Influence of the initial initial linolenic acid content

	$(tot\ 18:3)_{t=0}, \%$				
	9	10	11	12	13
Temperature (°C)	172	185	193	204	214
Time (h)	1180	368	178	73	33

At a given deodorization time and temperature we can consider the effect of initial fatty acid composition on the r ratio. It can be clearly seen in Table 25 that the influence of linolenic acid content is much stronger than that of linoleic acid. Rapeseed oils in Europe contain mostly only 8 to 10% linolenic acid. Raisio Margariini (Raisio, Finland) provided neutralised bleached rapeseed oil containing 11.9% linolenic and 21.9% linoleic acid. Calculating for this oil, 204°C and 74 hours were chosen to produce selectively isomerized oil as an acceptable compromise between the expected E fatty acid composition and the deodorization time realisable in pilot plant conditions.

Table 20

Effect of initial fatty acid composition on the r ratio

a. Linolenic acid

	$(tot\ 18:3)_{t=0}, \%$				
	9	10	11	12	13
Ratio r	7.6	8.5	9.3	10.1	11.0

$(tot\ 18:3)_{t=0} = 21\%$

calculated for 204°C, 73h

b. Linoleic acid

	$(tot\ 18:2)_{t=0}, \%$				
	19	20	21	22	23
Ratio r	11.2	10.7	10.1	9.7	9.3

$(tot\ 18:3)_{t=0} = 12\%$

calculated for 204°C, 73h

For the production of reference oil (“zero *trans*” oil) a 4.5-hour deodorization at 175°C was selected, at which no significant geometrical isomerization is expected. The pilot plant operations were performed according to the procedure detailed in chapter 3.1.2.

4.3.2.2. Quality of selectively isomerized and “zero *trans*” oils

Three batches of the selectively isomerized oil were produced, their *trans* fatty acid profile was very close to that predicted by the theoretical model (Table 26). The (*E*)-linoleic acid and (*E*)-linolenic acid content was 0.56 and 5.12% on average, which is close to the target values of 5.0 and 0.5%. The reference oil (seven batches were produced) did not contain detectable amount of (*E*)-isomer.

Table 21
(*E*) fatty acid content of selectively isomerized and “zero *trans*” rapeseed oils^a

	Temp.	Time	<i>trans</i> C _{18:2} , %		<i>trans</i> C _{18:3} , %	
	°C	h	meas. ^b	theo ^c	meas.	theo
Bleached oil			n.d. ^d	n.d.	n.d.	n.d.
SIO ^e , batch 1.	204	74	0.55	0.52	5.07	5.02
SIO, batch 2.	204	67.5	0.49	0.48	4.71	4.70
SIO, batch 3.	205	82	0.63	0.63	5.58	5.63
“Zero <i>trans</i> ” oil, average	175	4.5	n.d.	0.00	n.d.	0.05

^a (*tot* C_{18:2})_{t=0} = 21.9 % (*tot* C_{18:3})_{t=0} = 11.9 %

^b Measured from the pilot scale produced oil

^c Theoretical value calculated from the model

^d Not detected

^e Selectively isomerized oil

Concerning the organoleptic properties both the isomerized and reference oils had a neutral taste and odor. Table 27 shows that no remarkable change occurred in the tocopherol content after deodorization at 175°C for 4.5 hours (710 mg/kg was measured in the bleached oil and 700 mg/kg in the deodorized oil). An acceptable 15% decrease was detected during the long-term treatments at 204-205°C, where

the total tocopherol content dropped to 600 mg/kg on average. The transmittance values at 420 nm suggest a weak heat bleaching effect at 175°C/4.5 hours compared to the long term operations at 204-205°C.

Table 22

Quality parameters of selectively isomerized and "zero trans" rapeseed oils

	Temp. °C	Time h	Peroxide value meq O ₂ /kg	T ₄₂₀ ^a %	Tocopherols		
					α	γ	δ
					mg/kg		
Bleached oil			4.7	3.6	230	460	20
SIO ^b , batch 1.	204	74	0.1	73.4	159	397	18
SIO, batch 2.	204	67.5	0.3	71.3	174	409	19
SIO, batch 3.	205	82	0.2	74.6	161	380	17
"Zero trans" oil ^c	175	4.5	0.2	30.6	230	450	20

^a Transmittance at 420 nm

^b Average sample of seven batches

^c Selectively isomerized oil

4.3.3. Industrial applications

4.3.3.1. Prediction and control of the (*E*)-polyunsaturated fatty acid level in industrial deodorization

It is of importance to the vegetable oil industry to have a model available to predict the (*E*)-polyunsaturated fatty acid content of deodorized oils. There is a strong trend to decrease the amount of (*E*) fatty acid isomers and the model is a convenient tool to calculate the expected degree of isomerization for given deodorization conditions and to figure out the necessary deodorization conditions to meet certain target values of (*E*)-isomer content.

In the following calculations geometrical isomerization will be characterized by the degree of isomerization will be used as it is independent of the fatty acid

composition of the oil and more widely applicable conclusions can be drawn.

In Figure 39 and 40 the isomerization degree of linoleic and linolenic acid is plotted in function of deodorization time at different temperatures, considering the industrially applied time and temperature range.

Figure 28

Degree of isomerization of linoleic acid in function of time

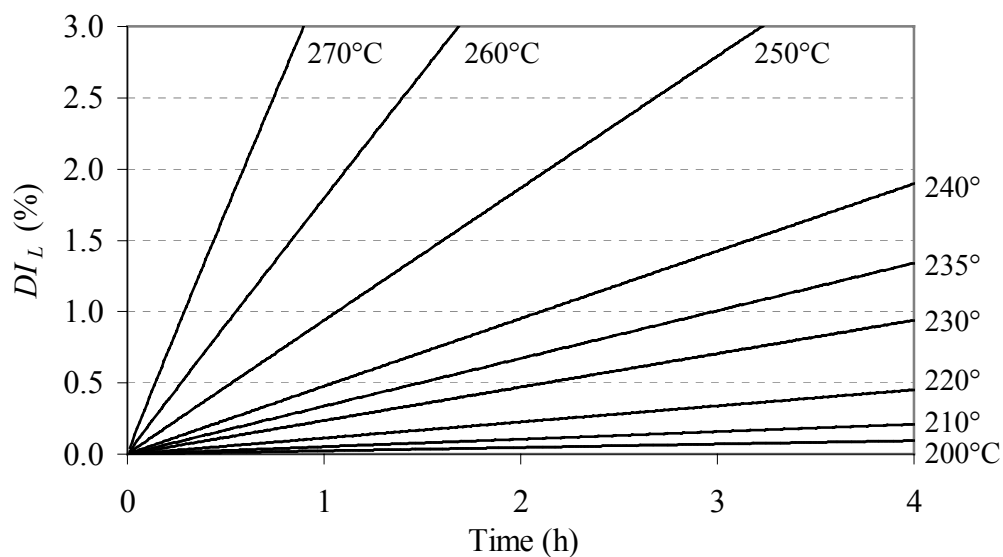
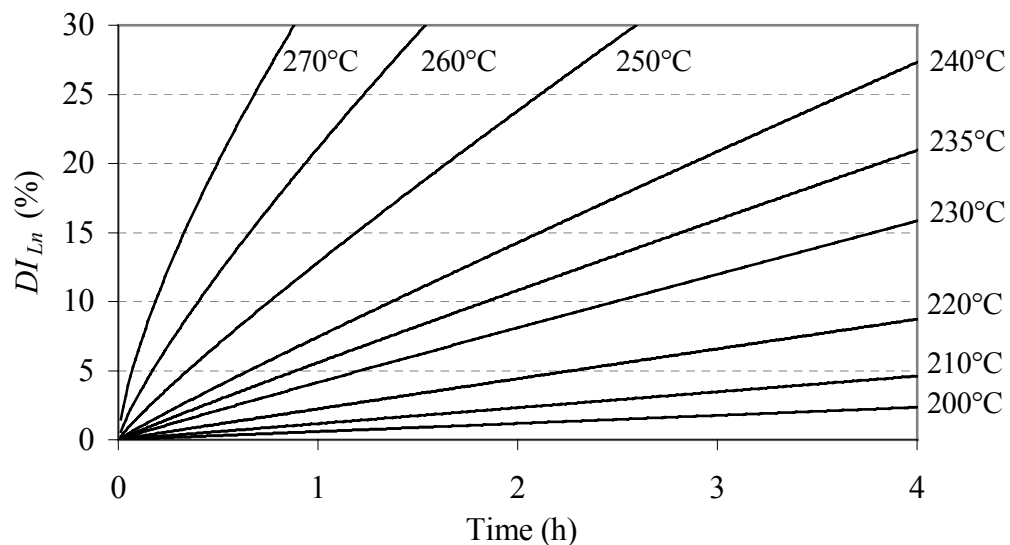


Figure 29

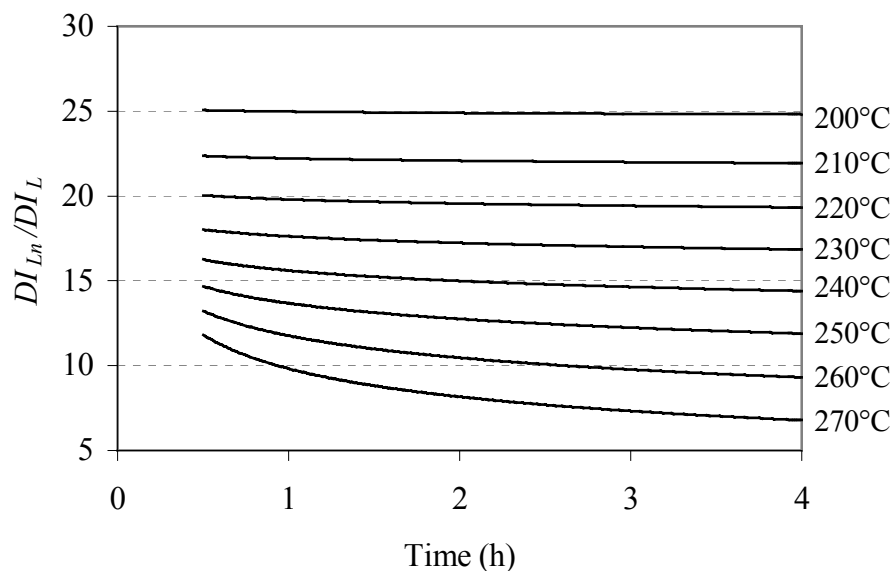
Degree of isomerization of linolenic acid in function of time



The temperature has stronger influence on the geometrical isomerization than the time of the operation, which agrees with the observation of Pudel and Denecke (1997) and Hénon et al. (2001). Under 230°C the isomerization degree of both linoleic and linolenic acid increases slowly, approximately in a linear function of time. A deodorization temperature of 235-240°C seems to be a critical point, above which both DI_L and DI_{Ln} increase very fast.

Figure 41 illustrates the changes in the ratio between the degrees of isomerization, DI_{Ln}/DI_L in function of deodorization time and temperature. Under the most generally applied conditions, DI_{Ln}/DI_L ranges between 12 and 18. It is in agreement with the observations of Wolff (1992) reporting a ratio of 13.4 ± 1.3 , independently from the nature and origin from the oil. Similarly, for regular deodorization conditions De Greyt (1997) reported a range of 12-14 on the basis of pilot plant experiments.

Figure 30
 DI_{Ln}/DI_L in function of the deodorization conditions



The figure above also suggests that the exact value of DI_{Ln}/DI_L strongly depend on

the temperature. At temperatures higher than 250°C DI_{Ln}/DI_L drops quickly with increasing time. In contrast, at temperatures lower than 240°C DI_{Ln}/DI_L is nearly independent of time. This makes it possible to estimate the probable deodorization temperature of an oil of unknown origin, simply by measuring the (*E*)-linoleic and (*E*)-linolenic acid content. When the measured DI_{Ln}/DI_L ratio is 13 or lower, it can be assumed that the oil was deodorized at least at 250°C.

In order to obtain refined oils with no significant quantity of (*E*)-isomer fatty acids, mild conditions have to be applied. Nevertheless the decrease of temperature has a limit, the principal goal of refining is the production of a blend and stable oil. Consequently, a reasonable compromise is necessary to optimise the industrial deodorization process. In the course of such optimization, the decrease of temperature has to be compensated by a longer deodorization time, during which sufficient heat bleaching, free fatty acid elimination and an efficient removal of odoriferous materials can be ensured.

There is no overall international regulation concerning the (*E*)-fatty acid isomer content of refined oils. According to the general practice, for sunflower oil maximum 1.0%, for rapeseed oil not more than 1.5% is accepted. In case of rapeseed oil these levels correspond to DI_L : 0.8-1.2% and DI_{Ln} : 14-15%, as far as sunflower oil is concerned the corresponding DI_L is approximately 1.5%. A further decrease in the amount of (*E*)-isomer fatty acids is still possible.

Taking into account the above considerations, Figures 42 and 43 could help refiners to control the geometrical isomerization during processing. The matching temperatures and deodorization times, applying which DI_L or DI_{Ln} meets a prescribed value can be easily determined. As the degree of isomerization is characterized in the above figures, those can be used for vegetable oils of any kind and origin. When expressing the total (*E*) fatty acid content as a function of deodorization conditions, the initial fatty acid composition of the oil has to be taken into consideration as well.

Figure 31
Calculation of deodorization conditions to keep DI_L at 0.5 and 1.0%.

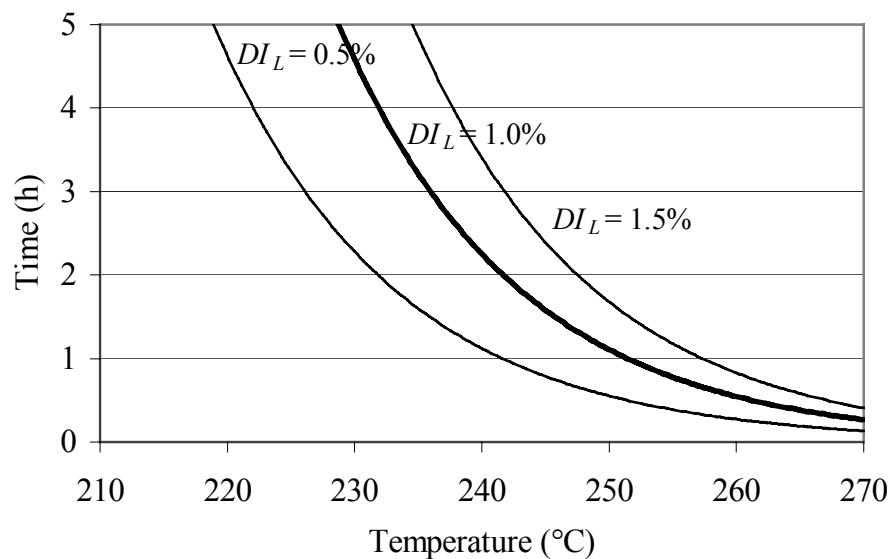
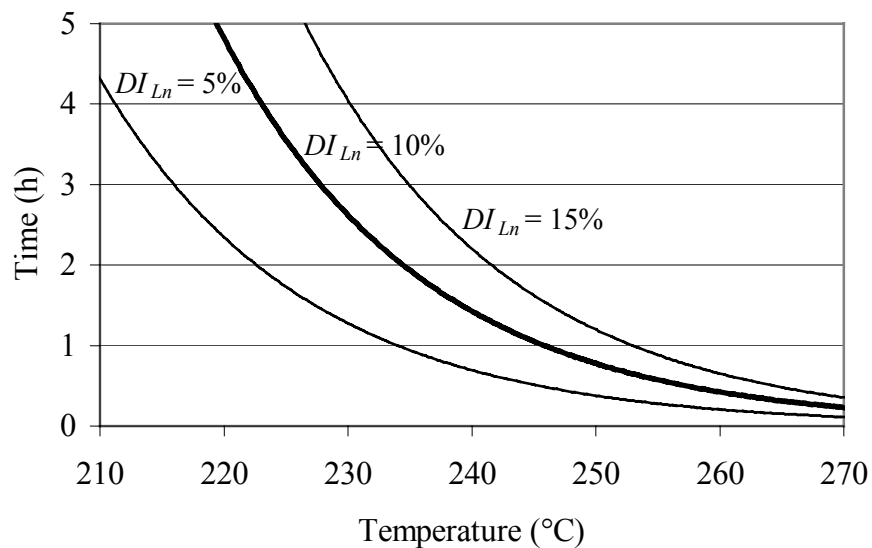


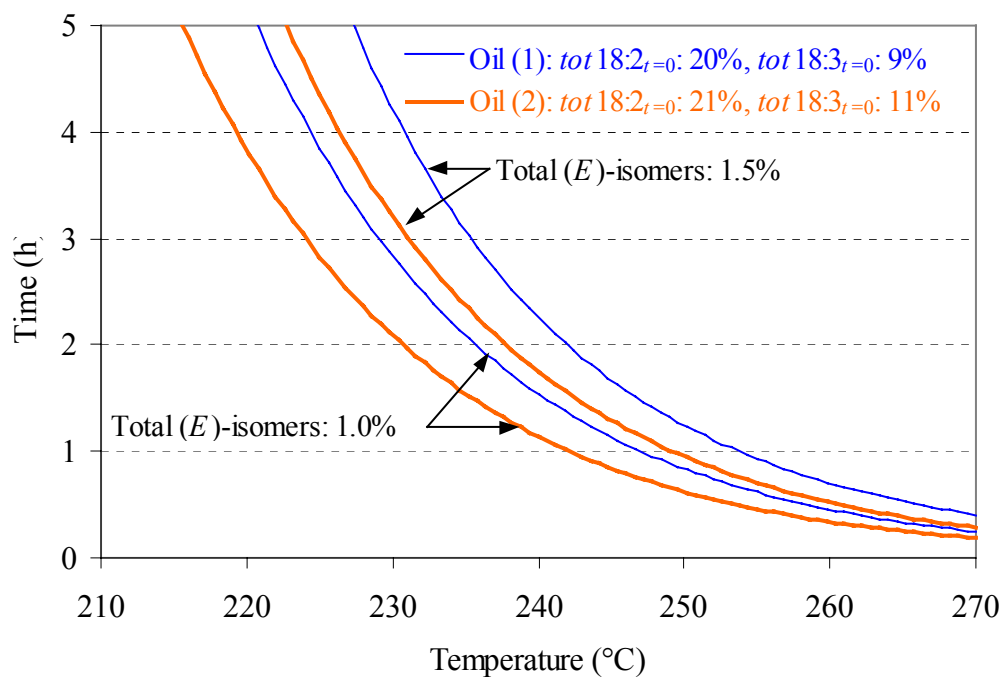
Figure 32
Calculation of deodorization conditions to keep DI_{Ln} at 5 and 10%.



In Figure 44 the conditions to limit the total (*E*) fatty acid isomer content to 1.5 and 1.0% are given for rapeseed oils, in case of different initial linoleic and linolenic acid content. The figure shows that in case of “oil (2)” deodorizing at 240°C for

about 100 min, a total (*E*)-isomer content of 1.5% is expected. To lower this value to 1.0% it is advisable to apply milder conditions such as 230°C, 120 min. Obviously, the influence of the initial fatty acid composition on the total amount of (*E*)-isomers is very important.

Figure 33
*Minimization of (*E*)-isomer content during rapeseed oil deodorization*



4.3.3.2. Survey of industrial deodorizers

The formation of geometrical isomers of linoleic and linolenic acids during the industrial process was checked in six deodorizers (marked D1 to D6) and it was studied if the equipment design has any effect on the (*E*) fatty acid content of the refined oils.

Sunflower, rapeseed and soybean oil samples were collected before and after deodorization. The samples were stored in refrigerator in glass vessels until analysis of (*E*) fatty acid content. All the samples were taken during stable operation of the

deodorizers under well-known conditions (temperature, duration, pressure and stripping steam dosage). In Table 28 the amounts of (*E*)-linoleic and (*E*)-linolenic acids formed during deodorization were compared with the theoretical values. Given the deodorization conditions and the fatty acid composition of the oils, the theoretical values were calculated applying Equation 24 and 25.

Table 23

*Survey of industrial deodorisers: Comparison of the measured and theoretical (*E*) fatty acid isomer content of refined oils*

Oil type	Deodorizer	Temp. °C	Time h	(<i>E</i>)-18:2, %		(<i>E</i>)-18:3, %	
				ind ^a	theo ^b	ind	theo
Sunflower	D1	200	1.5	0.04	0.02	-	-
Sunflower	D1	210	2.0	0.17	0.07	-	-
Sunflower	D1	227	1.5	0.16	0.19	-	-
Sunflower	D2	223	2.0	0.16	0.19	-	-
Sunflower	D2	223	2.0	0.15	0.19	-	-
Sunflower	D2	225	3.5	0.47	0.37	-	-
Sunflower	D3	223	2.0	0.16	0.19	-	-
Sunflower	D4	222	2.0	0.31	0.08	-	-
Sunflower	D4	225	2.0	0.74	0.21	-	-
Sunflower	D5	238	1.5	0.51	0.42	-	-
Sunflower	D5	240	1.5	0.60	0.48	-	-
Rape	D2	215	2.0	0.08	0.03	0.19	0.32
Rape	D2	220	3.5	0.08	0.09	0.57	0.70
Rape	D3	200	2.0	0.00	0.01	0.07	0.11
Rape	D3	226	2.0	0.05	0.08	0.43	0.57
Rape	D6	243	2.0	0.27	0.25	1.78	1.91
Rape	D6	246	2.0	0.35	0.30	1.99	2.06
Soya	D3	210	2.0	0.05	0.06	0.14	0.21
Soya	D3	226	2.0	0.20	0.19	0.48	0.59
Soya	D3	227	2.0	0.18	0.21	0.49	0.62
Soya	D4	225	2.0	0.82	0.18	1.47	0.52

^aMeasured from industrial sample

^bTheoretical values from the model

Good correspondence was found in case of five of the six deodorizers. The only exception was deodorizer D4, where the measured (*E*)-isomer level systematically much higher than predicted. For the above mentioned five equipment the measured (*E*)-linoleic acid content of 18 samples varied from 0 to 0.60%, the deviation from the theoretical values was insignificant (maximum: 0.12%, average: 0.02%). In case of the 3 samples from equipment D4 the measured (*E*)-linoleic acid content (0.31, 0.74 and 0.82%) significantly exceeded the corresponding calculated values by 0.23, 0.53 and 0.64% respectively.

Concerning linolenic acid, apart from deodorizer D4, the measured (*E*)-linoleic acid content of 9 samples ranged between 0.07 and 1.99%. The highest difference between the measured and calculated values was -0.13% (-0.11% on average). In case of equipment D4, the (*E*)-linoleic acid content of one single sample was measured (1.47%) and compared to the theoretical value a huge difference (0.95%) was found.

These results suggest that the deodorizer design (equipment D4) had an effect on the level of geometrical isomers. The possible causes could include local overheating, inhomogeneous heating, or liquid holdup (heterogeneous residence time) that may have resulted in a positive deviation from the model (a higher *DI* or (*E*) fatty acid content than the theoretical).

4.4. SURVEY ON (*E*) FATTY ACID CONTENT IN REFINED OILS FROM DIFFERENT COUNTRIES

First, Ackman et al. (1974) investigated the level of (*E*)-linolenic acid in North American oils. From the last decade the strong consumers' demand for edible oils with low (*E*)-isomer content forces refiners to take the (*E*) fatty acid formation more into consideration when optimizing the deodorization process. Extensive reports on

(*E*) fatty acid content of refined oils were published are available only from this period. Wolff (1992 and 1993a) studied edible oils sold in European countries (Belgium, France, Germany, Great Britain), de Greyt (1996) analyzed the products from the Belgian market. Refined oils purchased in the U.S. were characterised by O'Keefe et al. (1994), levels of (*E*) fatty acid isomers in oils produced in Mexico has recently been reported by Medina-Juárez et al (2000). In Table 29 the main results of the above investigations are overviewed.

Table 24
Level of (E)-isomer fatty acids in commercial edible oils

Reference	Country	Oils ^a	n ^b	DI_L %	DI_{Ln} %	Total <i>E</i> ^c %
Ackmann et al. (1974)	Canada	sb, rp	9	n.r. ^d	15.2-53.8	n.r.
Wolff (1992)	France	sb, rp, mix	8	0.2-2.2	2.3-29.6	0.3-3.5
Wolff (1993a)	Europe ^e	sb, rp, mix	12	0.7-2.6	10.5-26.9	1.8-3.3
O'Keefe et al. (1994)	U.S.	sb, rp, mix	16	0.3-3.3	5.9-37.1	0.6-2.8
De Greyt et al. (1996)	Belgium	sb, sf, co, pn	18			0.1-4.6
Medina-Juárez et al. (2000)	Mexico	sb	18	n.d.-3.5	11.5-22.3	0.9-2.9

^a sb: soy, rp: rape, mix: mixture of soy and rape, sf: sunflower, co: corn, pn: peanut oils

^b Number of samples

^c Total amount of *E* isomer fatty acids

^d Not reported

^e Including Belgium, Germany and Great Britain

In the present study, edible oils from different countries were analysed focusing on the total (*E*) fatty acid content and the degree of isomerization of linoleic and linolenic acids. The results were compared with those of the relevant studies of the last ten years. Different types of commercially available refined oils were purchased in supermarkets, in Austria (8 samples), Germany (10 samples), Poland (6 samples),

in Romania (7 samples) and in the U.S. (12 samples). Concerning Hungary, 13 imported and 15 locally refined oils were collected (Table 30). The 71 refined oil samples were divided into two groups according to their fatty acid composition. Sunflower, high oleic sunflower, corn, safflower and peanut oils were included in the oleic-linoleic group, the oils containing noticeable amount of linolenic acid (soy, rape, wheat germ and walnut oils) formed the linolenic group.

Table 25
Origin of samples for the survey of geometrical isomerization in edible oils

Country	Oleic-linoleic group					Linolenic group			
	Sunflower	High oleic sunflower	Corn	Safflower	Peanut	Rape	Soy	Wheat germ	Walnut
Austria	2		2			2	1	1	
Germany	1	1	2	1	1	3			1
Hungary i. ^a	9		1			2	1		
Hungary d. ^b	10					5			
Poland						6			
Romania	7								
U.S.	3					3	6		

^a imported oils

^b domestic production

4.4.1. (*E*) fatty acid content and degree of isomerization

Linoleic group

The total *E* fatty acid content in the oleic-linoleic group was lower than 1.0% and lower than 0.7% for 70 and 80% of the samples respectively. In most cases DI_L was lower than 1% but concerning the individual countries, important differences were noticed. As Table 31 illustrates, homogeneously low values were found in case of the Austrian, German and domestic Hungarian oils. For these countries mean values of 0.84 ± 0.15 , 0.70 ± 0.22 and $0.70 \pm 0.23\%$ were obtained respectively. To the

contrary, DI_L of the Romanian samples represented a wide range ($1.04 \pm 1.00\%$), visualised by the distribution profile of the DI_L values (Figure 45).

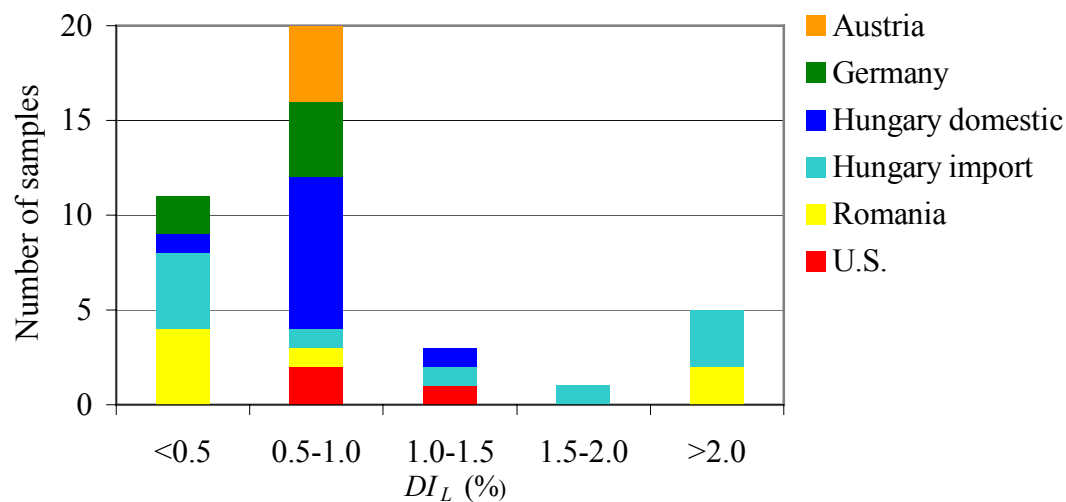
Table 26
Survey of (*E*)-isomer fatty acids in commercial edible oils

Country	n ^a	Total E ^b (%)	DI_L (%)	DI_{Ln} (%)	DI_L/DI_{Ln} (%)
<i>Oleic-linoleic group</i>					
Austria	4	0.57 ± 0.10	0.84 ± 0.15		
Germany	6	0.46 ± 0.30	0.70 ± 0.22		
Hungary, import	9	0.81 ± 0.58	1.27 ± 0.96		
Hungary, domestic	10	0.45 ± 0.15	0.70 ± 0.23		
Romania	7	0.65 ± 0.63	1.04 ± 1.00		
U.S.	3	0.63 ± 0.23	0.97 ± 0.44		
<i>Linolenic group</i>					
Austria	4	1.22 ± 0.61	0.98 ± 0.38	12.1 ± 6.4	12.1 ± 3.2
Germany	4	1.17 ± 0.51	0.68 ± 0.22	9.0 ± 1.9	14.1 ± 3.9
Hungary, import	4	1.85 ± 0.70	1.27 ± 0.60	16.9 ± 6.6	13.7 ± 1.4
Hungary, domestic	5	1.28 ± 0.11	1.05 ± 0.16	13.6 ± 1.6	13.0 ± 0.8
Poland	6	0.81 ± 0.15	0.55 ± 0.14	7.3 ± 1.3	13.8 ± 2.6
U.S.	9	1.63 ± 1.30	1.30 ± 1.30	13.8 ± 11.5	11.3 ± 1.5

^a Number of samples

^b Total amount of (*E*)-isomer fatty acids (mean ± SD)

Figure 34
 DI_L of commercial refined oils (sunflower, high oleic sunflower, corn, safflower and peanut oils)



A huge difference was found between the locally refined and the imported oils in

Hungary. Comparing to the domestic oils, the (*E*) fatty acid content of the imported oils was nearly two times higher, DI_L was 1.3% on average and distributed in a broad range. In case of the American samples DI_L ranged around 1.0%.

Linolenic group

The total (*E*) fatty acid content in linolenic group is summarized in Table 26. The lowest values with very low standard deviation were found in Poland ($0.81\pm 0.15\%$). The average values of the Austrian, German and domestic Hungarian samples ranged similarly between 1.2 and 1.3%. The highest means were observed in the Hungarian import and in the American oils (1.85 and 1.63%). The well known differences between the deodorization conditions in Europe and in the U.S. results in higher amount of (*E*)-isomers in the American refined oils. Also as a consequence of the high temperature treatment, the lowest DI_{Ln}/DI_L ratios ($11.3\pm 1.5\%$) were found in these samples.

The distribution of the degree of isomerization for the linolenic type oils is summarized in Figure 46 and 47.

Figure 35

DI_L of commercial refined oils (rape, soybean, wheatgerm and walnut oils)

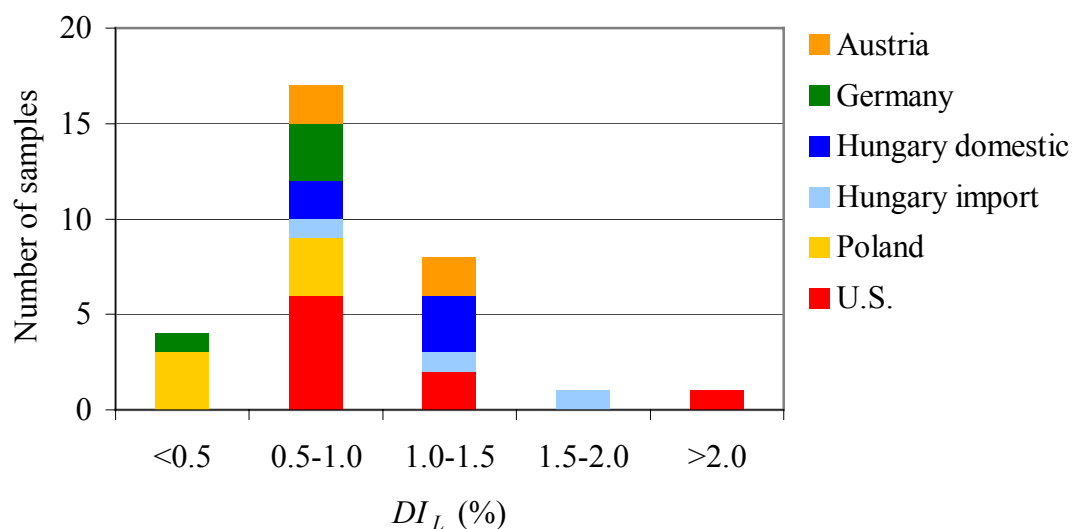
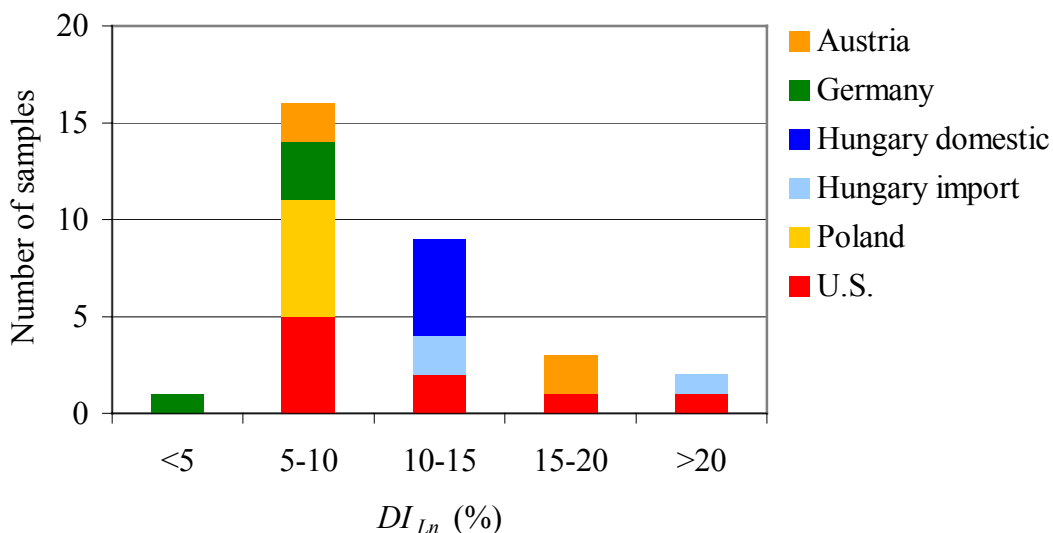


Figure 36

DI_{Ln} of commercial refined oils (rape, soybean, wheatgerm and walnut oils)



In case of the U.S. samples DI_L and DI_{Ln} ranged from 0.5 to 4.7% and from 6.3 to 43.2% respectively, which are very similar to the ranges reported by O’Keefe (DI_L : 0.3-3.3%, DI_{Ln} : 5.9-37.1%). The degrees of isomerization of the Polish and German samples were fairly low ($DI_L < 0.7\%$, $DI_{Ln} < 10\%$ on average).

Taking into account all the 71 samples, we can conclude that three-quarter parts of the oils showed DI_L lower than 1%, and about halve of the samples had DI_{Ln} lower than 10%. This suggests a pronounced decrease in the isomerization level of refined oils during the last decade. In the main studies carried out between 1992 and 1994 (36 samples), approximately one-third part of the oils showed $DI_L < 1\%$ and only 14% of them had $DI_{Ln} < 10\%$.

5. SUMMARY

Formation of geometrical isomers of polyunsaturated fatty acids during prolonged heating was investigated.

A kinetic model of geometrical isomerization, taking into account the degradation of fatty acids was established at laboratory scale. No degradation of linoleic acid was observed even during 48-hour heating at 230°C, but a significant decrease in total linolenic acid content was found. Both the degradation and isomerization reaction followed first order kinetics. The rate constants of degradation and geometrical isomerization (k_{dLn} , K_{iL} and K_{iLn}) as well as their dependence on temperature was determined. Such complete description of these rate constants has not been published yet. The results obtained in the laboratory were confirmed by pilot plant experiments. Therefore, the model provided accurate information on the kinetics of the studied reactions.

Investigating the distribution of the individual geometrical isomers of linolenic acid, four compounds were detected. The two major isomers were (9Z,12Z,15E)- and (9E,12Z,15Z)-linolenic acid and the two minor components were (9Z,12E,15Z)- and (9E,12Z,15E)-linolenic acid. The *E,Z,E* isomer forms in a two-step reaction at the expense of the two main components. Concerning linoleic acid, two geometrical isomers were identified: (9Z,12E)- and (9E,12Z)-linoleic acid. Strong linear correlation was found between the degree of isomerization and the relative amount of the individual isomers, limited to a range $DI_{Ln} \leq 35\%$ and $DI_L \leq 2.1\%$. In case of linolenic acid the relative probability of formation of the *Z,Z,E*-, *E,Z,Z*-, *Z,E,Z*- and *E,Z,E* isomers at the beginning of the reaction was 51.5, 43.6, 4.9 and 0% respectively, showing that the double bond in position 15 is the most prone to geometrical isomerization. No corresponding data have been reported for linoleic acid. In the present study the initial probability of formation of the *Z,E* and *E,Z* isomers was 63.2 and 36.8% respectively.

The kinetic model was successfully applied both in the field of research and industrial deodorization. In an international nutritional study (“Nutritional and Health Impact of *Trans* Polyunsaturated Fatty Acids in European Populations”) a refined oil with 5% (*E*)-linolenic acid and maximum 0.5% (*E*)-linoleic acid was requested, which can not be achieved under regular process conditions. Apart from this unusual amount and composition of geometrical isomers, the oil had to meet the general refined oil specifications. Using the model, deodorization conditions of 74 hours at 204°C were chosen as an acceptable compromise between the expected profile of geometrical isomers and the deodorization time realisable in pilot plant conditions. The selectively isomerized rapeseed oil produced in 100-liter batches met the quality expectations.

Concerning the industrial applications, the model showed that under regular deodorization conditions, the decrease in the total linolenic acid content is not noticeable. The model provides a useful tool to the oil refiners to minimize the geometrical isomerization during deodorization. Applying regular deodorization parameters, isomerization of linolenic acid is 12-18 times faster than that of linoleic acid. The selectivity of the reaction (K_{iLn}/K_{iL}) decreases with increasing temperature. Ranges of temperature and time were calculated, applying which the degree of isomerization (or the (*E*)-isomer fatty acid content) can be kept under a prescribed value.

Deodorizers can be characterized from the point of view of geometrical isomerization, by comparing the (*E*) fatty acid contents measured from the deodorized oil and calculated by using the model. In a survey of industrial deodorizers, in case of one of the six equipment a systematic positive deviation from the theoretical values was found. This deviation was associated with local overheating and/or heterogeneous residence time in the equipment.

Analysing the fatty acid profile of refined oil of unknown origin containing both linoleic and linolenic acids, the applied deodorization temperature can be estimated

by considering the total amount of geometrical isomers, the ratio of DI_{Ln}/DI_L and the distribution of the individual isomers. No important quantity of geometrical isomers can be detected ($\sim 0.1\%$) after deodorization at 190°C during 4 hours. Presence of only the two main geometrical isomers of linolenic acid (Z,Z,E and E,Z,Z) indicates a mild deodorization possibly at a temperature lower than 220° . Detectable amount of E,Z,E linolenic acid isomer appear at about 230° after 2-hour heating, its relative percentage grows with increasing temperature. Presumably high deodorization temperature ($>250^\circ\text{C}$) was applied when DI_{Ln}/DI_L in the deodorized oil is lower than 12.

In order to get up-to-date information on the level of geometrical isomerization in refined vegetable oils, 71 commercially available refined oil products from European countries and from the U.S. were analysed. Three- quarter parts of the samples showed DI_L lower than 1% and about halve of the oils had DI_{Ln} lower than 10%. Comparing with the data available from the period of 1992-1994, the level of geometrical isomers in refined oils indicates a decreasing tendency.