Biotechnology and fatty acids: new perspectives for agricultural production?

Proceedings of a discussion meeting organized by
the Division of Industrial Microbiology of Wageningen Agricultural
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Preface

Agriculture in the Netherlands and within the EC presently faces the problem of surplusus. Consequently, new directions for agriculture and new outputs from agriculture not only for food purposes but especially for the non-food industries are urgently required. Better links are needed between agriculture as supplier of raw materials and industry producing from these renewable materials. Such more market-oriented agricultural production within the EC is expected to have various fields of opportunities. The production of oleochemicals has, however, special promise because the wide structural diversity of oils and fats makes them suitable for various industrial production processes.

To highlight these opportunities in oleochemicals for Dutch and for EC-agriculture, a discussion meeting 'Biotechnology and fatty acids: new perspectives for agricultural production?' was organized by the Division of Industrial Microbiology of Wageningen Agricultural University and by the Agrotechnological Research Institute, Wageningen. The aim of the meeting, held on 26 April 1989 in Wageningen, was to bring together experts on fats and oils from industry, from research institutes and universities, and from policy-making bodies in order to discuss the possibilities of an integrated research approach. Ten scientists with various backgrounds gave their views on specific aspects of fatty acid production by plants and micro-organisms as well as on the utilization of fatty acids both in the food industry and in the chemical industry.

The meeting attracted 150 participants, mainly from the Netherlands. It was opened with introductory lectures by A.H. Eenink on behalf of the Agrotechnological Research Institute and by J.A.M. de Bont on behalf of the Division of Industrial Microbiology. The closing address was by J.J. Groeneveld of the Dutch Ministry of Agriculture and Fisheries.

The present proceedings contain abstracts of the lectures of the expert speakers. The abstracts have been prepared by students attending the meeting and subsequently M. Trinn patiently corrected them. M.J.E. Boleij-Schuijt deserves special thanks for her work in preparing the manuscripts for this volume and J.H.W. van der Schild for his work in co-organizing the meeting.

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OUTLOOK FOR OLEOCHEMICALS IN EUROPE

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Actions in the field of biotechnology and agricultural related research within the European Communities are realized at three different levels by the Directorate General XII (Science, research and development).

1. The basic research

Included in the basic research level activities are the "Biotechnology Action Program" (BAP) and its follow up programme "Biotechnology Research for Innovation, Development and Growth in Europe" (BRIDGE) which is now prepared.

2. The precompetitive research

The precompetitive research level is represented by the programs ECLAIR and FLAIR. The latter is mainly concerned with food, whereas ECLAIR is more intended for industrial applications. If we consider the oleochemical industry, the precompetitive research program includes research and development work in the field of renewable resources with the cooperation of universities, institutes and also agro-industrial companies. A list of approximately fifty oil plants has been compiled to be considered in these programmes. In Table 1 some of the most important plants are given together with the typical fatty acids they contain.

3. The demonstration

The purpose of the work at the demonstration level is to carry out actions aimed directly at applications. Therefore, it is performed on a much larger scale and it mainly includes cooperations between industrial and agricultural companies. It should also comprise rentability and reliability studies for new products and/or new technologies. Some of the more promising oil plants involved in the working programmes at this level are given in Table 2.

Table 1. Important oleaginous plants and some typical fatty acids

Plant	Typical fatty acids
Alliaria petiolata	erucic and linoleic
Brassica alba	oleic and erucic
Brassica juncea	erucic, oleic and linoleic
Calendula officinalis	calendulic and linoleic
Camelina sativa	linoleic
Carthamus tinctorius	oleic and linoleic
Coriandrum sativum	petrocelenic
Cucurbita pepo	oleic and linoleic
Cuphea	lauric, capric and caprilic
Eruca sativa	erucic and oleic
Euphorbia	vernolic
Foeniculum vulgare	oleic and petrocelenic
Lesquerella	3-hydroxy fatty acids
Limnanthes alba	erucic
Lunaria annua	erucic and oleic
Madia sativa	oleic and linoleic
Papaver somniferum	linoleic
Raphanus sativus	oleic and erucic
Stokesia laevis	epoxy fatty acids
Thlaspi arvense	erucic, linoleic and linolenic

New results obtained through plant breeding do not have to be demonstrated like in the cases of rape and sunflower because of the existing high levels of agrotechnology. In certain other cases as for instance with flax or pea only some process engineering aspects have to be adapted, but for many other plants the whole agricultural technology has to be developed. In these cases demonstration projects are really needed. As far as these programmes are concerned the Directorate is responsible for the organization of cooperations, for selecting projects, for establishing project groups, for deciding on financial support etc. Much emphasis is placed on workshops and meetings to get together experts in different fields.

Table 2. Important oleaginous plants for demonstration studies

Plant	Industrial application
rape and mustard	erucic acid and animal feed
high oleic sunflower	oleic acid and animal feed
castor	castor oil

Common agricultural policy within the EC

The Directorate-General XII is not responsible for the common agricultural policy, however some indications are necessary in order to allow a general assessment of the oleochemical sector. First it is useful to get an idea about the total turnover (production and utilization) of fats and oils within the 12 member countries (Scheme 1). Numbers given in scheme 1 are in million tons and are related to the year 1987. The figures in the scheme show that more than 80% of the utilization is based on imported products, mainly from "third countries", for some of them certain of the countries have recently taken measures to protect their own industries. This of course creates some difficulties for the EC industries. It needs also to be mentioned that oilseeds are available to EC industries at world market level (no levy at entry) and that therefore a deficiency payment is foreseen to farmers. The system provides for maximal quantities.

Examples for demonstration:

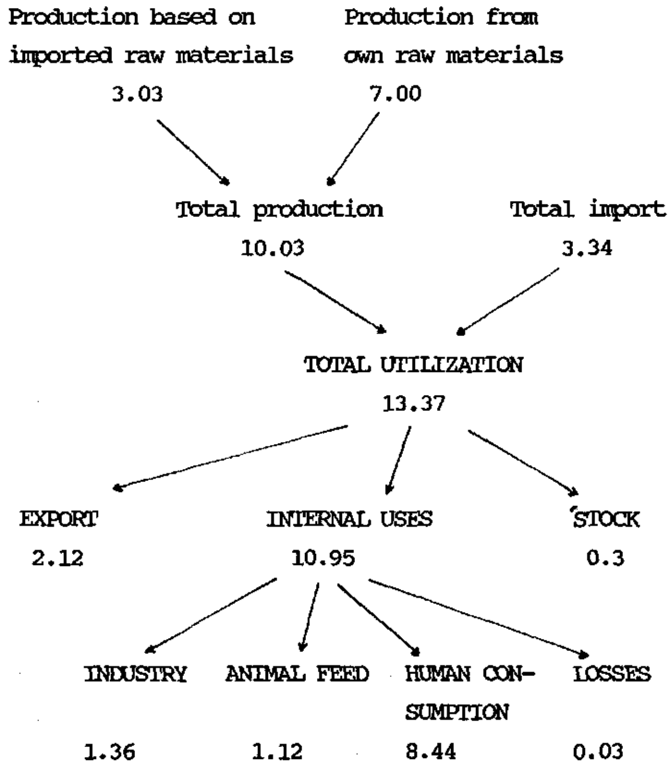
The production of Castor oil is very promising because there are good results on small plots available, and the marketing possibilities seem to be good.
A strong interest from the industry exists in high erucic rapeseed oil which may lead to a doubling of the surface used for this purpose.

Apart from the evident benefits, the new developments in producing high oleic sunflower oil raise some problems as well. The main point is that the seed market in Europe is dominated at present by a U.S. firm which through a contracting system takes back all seed produced by the farmers. Another problem might be the competition with tallow for the purpose of obtaining oleic-acid and overmore the replacement of tallow would lead to environmental problems if no possibility of disposal can be found.

Cultivation of linseed has strongly declined over the past decades within the Community and the production of linseed oils is now nearly fully based on imported quantities.

It can be concluded from the above that there is a positive outlook for oilplants on the condition that they are tailor-made for the needs of industry and their price is competitive to the main concurrent petrol.

Scheme 1. Turnover of fats and oils in the EC (million tons in 1987).



COORDINATION OF AN INDUSTRY-ORIENTATED AGRICULTURE IN THE FEDERAL REPUBLIC OF GERMANY

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The task of the German Association of Chemical Industries (Verband der Chemischen Industrie, VCI) is to coordinate in the development of renewable resources. This coordination includes two main areas:
- production of oils and fats from "home" -EC industrial crops
- utilization in larger quantities of carbohydrates originating from starch and hopefully from cellulose in the chemical industry.

In the present presentation, the production of oils and fats will be discussed.

After the second oil crisis in 1979, the VCI decided to form a committee with the following goals:
- to look for alternative and supplementary resources for mineral oils
- to find new outlets for agricultural production that would be relevant to industrial needs outside the food industry
- to overcome environmental problems by the use of oils from crops since these plant oils are generally biodegradable.

In order to be successful with the intended programme, the earlier frame of coordination between universities, institutes, agriculture and industry had to be changed. A reorientation - now especially considering the industrial aspects - had to be realized. Three main directions were considered:

- Agricultural policy and its market regulations.
  These regulations are more important in the food sector than in the industrial sector.

- Plant breeding of crops to yield tailor made products for the industry.
  A very good example for the need of such a change in research and development strategies is rapeseed. Earlier, plant breeding was oriented exclusively to develop "zero-zero" rapeseed for food purposes whereas now, high erucic rapeseed is aimed at for applications in the chemical industry.

- Formulation of new subjects for research and development.
  At this point cooperation with universities is important. New ideas for the conversion of material derived from new, industrial plants are necessary. Therefore, the VCI has to coordinate cooperation between research institutes, universities, the chemical firms, agricultural organizations and the oil mill industry.

As a first result, projects between several companies were established for the conversion of traditional vegetable oils to new products in the oleochemical industry. These projects, the "German oleochemical research program" are fully financed by the Federal Ministry of Research and Development. This programme involves very precise and concrete projects to be carried out in universities for fundamental research on new processes based on oils and fats to evaluate chemicals with new qualities in direction of biodegradability, bioactivity and biocompatibility. A second programme was established on the plant breeding side with as participants apart from the plant breeders the chemical industries and farmers associations. This programme is also discussed with the EC Commission and with the national ministries of Agriculture and of Research and Development.

The oleochemical research programme

The start of the coordination work may be traced back to a symposium on oleochemicals held in the autumn of 1985 where the state of the art, gaps in research and development, and interests on the sides of universities institutes and industries were discussed. It was concluded that both the industry and the university sides did need considerable research and cooperation was considered to be urgent. The working group Fundamental Research Oleochemicals was set up with the participation of the chemical companies Henkel, Schering, Hoechst, Unichema and Stockhausen and from the university side the Technical University of Braunschweig, Society of Biological Research Braunschweig, University of Oldenburg, Technical University of Aachen, University of Münster, University of Duisburg, and the University of Bayreuth were ready to participate. Project teams were set up and these teams suggested to perform research to be supported financially by the Federal Ministry of Research and Development in the following areas:

- biotechnological processes
- catalytic processes
- functionalizing and C-chain linking
- oleochemical analytics.

Groups were set up in order to streamline the projects in the sense of the policy of the Ministry of Research and Development and to fit the research in general schemes of work on renewable resources. Regularly, symposiums have been organized to discuss results and problems in detail and further on to decide on continuation, modification or - evetually- on finishing a project or on starting new ones.

The plant breeding programme

The VCI is also responsible for cooperation in the field of industrial plant breeding for new oil products and for the connected marketing. The most important areas are:

- high erucic acid rapeseed oil
- high oleic acid sunflower oil
- high linoleic acid linseed oil
- high linoleic acid castor oil oil.

The various projects are summarized in Table 1. Below, two examples on erucic acid and on oleic acid, respectively, will be discussed in more detail.

1. <u>"High erucic" rapeseed</u>

Interests in the past for research and development and connected subsidies were in the direction of "zero-zero" rapeseed. At present- because of industrial applications - a high erucic acid content together with a high yield and a low glucoseinolate concentration is desirable. The development is based on "old" crop species imported mostly from Eastern European countries having an erucic acid content of approximately 45 %. An increase up to 60 or 80 % (60 % is a more realistic limit)

Table 1. Projects on oleaginous crops.

CROP	BREEDING	CULTIVATION	APPLICATION	MARKET	REALIZATION
Rapeseed	High erucic acid content (>60%) Yield increase Decrease of glucoseinolates	Difficulties in distinguishing between erucic-poor and erucic-rich rape	Antifoam Additive in mineral oil and plastic processing	Approx. 30.000 t EC-market	Short term
Linseed	High linoleic acid content Yield increase	Improvement of harvest techniques	Lacquer Lino	100.000 t EC-market	Short term
Sunflower	High oleic acid content Yield increase	No special problem	Detergents	Several 100.00 t EC-market	Short term
Castor	Yield increase	Improvement of harvest techniques	Fibres	Approx. 80.000 t EC-market	Short term
Cuphea	Domestication	Not yet realistic	Detergents	Large quantities	Very long term (10 years)

might be achieved. For this purpose, the Ministry of Agriculture promotes the cooperation between the oil mill industry, the chemical industry and agriculture. In a three year test programme cultivation is done to study harvest techniques and processing methods.

The chemical industry is now ready to make contracts with agriculture and the oil mills for the delivery of about 10 000 ton/year high erucic acid rape seed oils. Prices are based on the world market prices for rapeseed oil plus a premium of 8 DM/100 kg for every percent of erucic acid up till 47 %. This system is of interest to all partners. The industry is interested in tailor made crops, the agriculture has the impression that they do not just get subsidy but are now in a new kind of partnership between agriculture and industry to produce products with additional value.

2. "High oleic" sunflower

The oleochemical industry is very much interested in products based on oleic acid. Until now, tallow containing about 30 % of oleic acid is imported from elsewhere for industrial purposes. Although its price is quite low, the processing costs greatly contribute to the total production costs of oleic acid from this source. "High oleic" sunflower species have already been developed - reaching an oleic acid content of about 80 % - which may serve as economic substitutes of tallow. The right prices for this sunflower oil is presently a matter of discussion but a similar stimulation system as that for high erucic acid rapeseed is going to come into existence.

These two examples on erucic acid and oleic acid show that the coordination for an industry-orientated agriculture in the Federal Republic of Germany is successful. However, this work is not restricted to Germany. Similar strategies with similar tasks have been worked out within the EC countries to eventually arrive at new processes and new products based on EC home made, tailor made oleochemicals.

Mr. Wittmeyer invites the Wageningen Agricultural University to cooperate with CEFIC-Commission Renewable Resources so that these results can be introduced to the activities of the EEC-Commission.

HEALTH EFFECTS OF DIETARY FATTY ACIDS

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More than 80% of fats and oils is used for human consumption. The health effects of fats and oils are becoming a more and more important determinant of consumers purchasing behaviour and in that way nutritional properties also determine production. During this presentation three aspects will be dealt with. The basic structure of fatty acids, the health effects of fatty acids and avenues for future research in the field of edible oils.

1. The structure of fatty acids

Fatty acids are consumed in esterified form, usually as triglycerides. The position of the fatty acids on the glycerol is a neglected area in dietary and nutrition research but future research in this field may be promising. The fatty acids can be classified according to the length of their C-chain (n) and the number of double bonds (Table 1). It should be noted that because elongation of fatty acids occurs at the COOH terminus nutritionists use a numbering system opposite to that usual in chemistry: the methyl terminus is counted as No. 1.

The human body is able to convert fatty acids in two different ways:

- shortening and elongation of the chain length
- addition of double bonds but only from the n - 7 position upwards.

This means that the food of humans has to contain certain fatty acids with double bonds below the n-7 position because they are precursors of the prostaglandins. The geometry of the double bond (Fig. 1) is also important because it determines the metabolic properties. In nature most unsaturated fatty acids occur in cis-isomeric form but hydrogenation (hardening) of oils often leads to isomers with the trans-isomeric form that have fully different physiological effects.

Table 1. Important fatty acids.

Fatty acid	Schematic structure 1 3 6 9 12 15 18 22 HCH COOH	Chain length and no. and position of double bonds (n)
<u>Saturated</u>		
lauric acid	#----------#	C 12 : 0
myristic acid	#------------#	C 14 : 0
palmitic acid	#--------------#	C 16 : 0
stearic acid	#----------------#	C 18 : 0
<u>Monounsaturated</u>		
oleic acid	#-------=-------#	C 18 : 1(n-9)
erucic acid	#-------=-----------#	C 22 : 1(n-9)
<u>Polyunsaturated</u>		
linoleic acid	#---=--=--------#	C 18 : 2(n-6,9)
α-linolenic acid	#-=--=--=-------#	C 18 : 3(n-3,6,9)
arachidonic acid	#---=--=--=--=----#	C 20 : 4(n-6,9,12,15)
eicosapentaenoic	#-=--=--=--=--=---#	C 20 : 5(n-3,6,9,12,15)
docosahexaenoic	#-=--=--=--=--=--=---#	C 22 : 6(n-3,6,9,12,15,18)

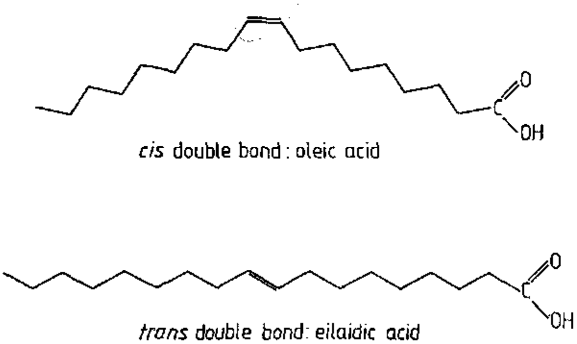

Figure 1. Structure of <u>cis</u> and <u>trans</u> fatty acids.

2. <u>Health effects</u>

2.1. Cholesterol and coronary heart disease

The major cause of death in most developed countries is myocardial infarction as the result of blocked coronary arteries. The obstruction in the arteries contains a yellowish material consisting mostly of cholesterol. Cholesterol (Fig. 2) is not a fatty acid but a sterol, but for reasons that are not quite clear the fatty acids that we eat strongly influence the cholesterol concentration in the blood. Since cholesterol is very poorly soluble in water, it is transported in the blood in special particles lipoproteins, the major one being LDL (low density lipoprotein).

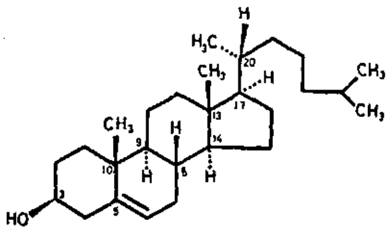

Figure 2. The structure of cholesterol.

Most of the cholesterol in lipoproteins is esterified with fatty acids, mostly linoleic acid. These cholesteryl esters form a hydrophobic core which is coated with phospholipids and amphophilic proteins and so it is transported to the place where it is needed. If the concentration of LDL in the blood is too high, cholesterol is deposited in the arteries which leads to obstruction and causes coronary heart disease. From experimental work, it is known that certain fatty acids raise the chance of getting a myocardial infarction because they enhance the concentration of cholesterol in blood.

The effect of certain fatty acids in the diet on the concentration of cholesterol in blood serum may be predicted according to an empirical formula derived by Keys from a large number of experimental observations:

$$\Delta \text{ Serum cholesterol (mmol/L)} = 0.03\ (2\ \Delta S - \Delta P) + 0.08\ \Delta\ (\text{chol})^{0.5}$$

chol	: cholesterol consumption (mg/megajoule)
S	: lauric + myristic + palmitic acid (% of energy)
P	: polyunsaturated fatty acids (% of energy)

The three saturated fatty acids and especially palmitic acid are the main food components that increase the serum cholesterol concentration and thus endanger the arteries. Average consumption of these three saturated fatty acids in the Netherlands is 30-40 g/day. They are mainly obtained from dairy fat, other animal fats and plant fats. Fatty acids with shorter chain length (C-6, C-8, C-10) or with a longer C-chain (C-18, stearic acid) do not influence the serum cholesterol level. There is now a strong tendency in U.S.A. and elsewhere to reduce the amounts of lauric, myristic and palmitic acid in foods. This trend causes of course major problems for the dairy and livestock industry, and in agricultural sectors such as the tropical oil plants, for they must find alternative products to replace these undesirable fatty acids.

2.2. Obesity

Obesity is a major social and health problem, and the feeling that it is mainly caused by fatty food, is rather widespread. In fact, experimental evidence in this field is very limited. The question whether a high fat diet (40 % of the daily calories as fat - the usual amount in affluent countries) causes more obesity than a low fat, high carbohydrate diet (30 % daily calories as fat - generally advised as more healthy) is still not settled.

2.3. Essential polyunsaturated fatty acids and prostaglandins

This is a new and challenging field. The substances playing a crucial role here are called eicosanoids which are substances with 20 carbon atoms. Eicosanoids, as for instance prostaglandins and leucotrienes, form a class of physiologically active substances. They are hormone-like compounds produced and acting locally. All eicosanoids are derived from dietary polyunsaturated essential fatty acids. According to their origins, they form two series (cf. Table 1):

- the n - 6 series is derived from arachadonic acid
- the n - 3 series is derived from eicosapentaenoic acid

Since the human organism cannot introduce double bonds at the n - 3 or the n - 6 position, a deficiency in these essential dietary fatty acids causes deficiency in these essential local hormones. But their overproduction may also lead to negative physiological effects.

The main effects of prostaglandins and leucotrienes are:

- Pain and inflammation. Taking Aspirine for example reduces the production of prostaglandins and thus reduces pain.

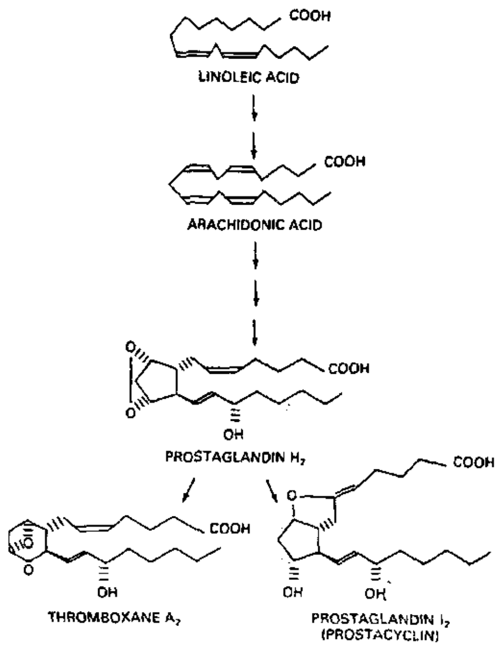

Figure 3. Prostoglandins from unsaturated fatty acids.

- Changes in the tension of smooth muscles. The tension of the arteries is effected, thus causing changes in the blood pressure. Prostaglandins influence the uterus, and thus they may be used as abortion pills to drive out the foetus. They also affect the lungs leading to asthma-like reactions.
- Blood clotting and blood platelet aggregation. This phenomenon is influenced by fatty acids in the food. Fish oils might reduce the risk of heart disease by stimulating the production of certain prostaglandins that inhibit blood clotting and thrombosis.
- Immunological reactions. These may also be affected by prostaglandins.

4. Speculations about avenues for future research on oils

In addition to the well known diet and cholesterol field, there is a whole new field where various exotic fatty acids become interesting from a pharmacological and pharmaceutical point of view. The fish oils mentioned above are one example; evening primrose oil, rich in C18:3 n-6 (γ-linolenic acid) is another. Progress is limited by the availability of interesting fatty acids (as triglycerides) in sufficient amounts and purity for human experimentation. Interesting compounds that come to mind are palmitoleic acid (C 16 : 1 (n-7)) and various n - 3 polyunsaturates.

Factors to consider for further research for successful food oils:

- Oils low in saturated C 12, C 14 and C 16 fatty acids have a marketing advantage because they do not raise serum cholesterol. For instance, low erucic acid rapeseed oil is already a great success.
- Oils high in a balanced mixture of various unsaturates may become prefered to oils that are high only in linoleic acid. Again, the new rapeseed oil containing all three classes: n - 9 (oleic), n - 6 (linoleic) and n - 3 (α-linolenic) is attractive from a nutrional point of view.
- Certain oils high in specific exotic fatty acid may have applications in the nutritional and/or pharmaceutical field. Oils with relatively high γ-linolenic acid content (e.g. Evening Primrose oil) are marketed with great success for the prevention and treatment of various ailments, even though hard evidence for effectiveness is lacking.
- The replacement of cholesterol-raising saturated fats and oils puts the food industry in a difficult position because saturated fats provide certain properties (structure, melting range, mouth feel) needed for the product. Any solution here that fulfills both technological and nutritional requirements could turn out to be very profitable.
- Fats and oils low in calories such as sucrose polyester are considered very promising; however, their consumer acceptance may be hampered by real or perceived side effects.

All in all, upgrading of edible oils so as to improve their nutritional properties is a promising area for the oil industry.

EDIBLE OILS: REFINING AND MODIFICATION PROCESSES

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Edible oils are extracted or expelled from seeds (e.g. sunflower seed or soya bean) or rendered from animal fat tissues as tallow or lard. The most important aspects of properties of crude oils in view of further processing are:

- taste (positive in native form in the case of olive oil, negative in most other cases)
- taste stability (mostly influenced by oxidation)
- colour
- safety factors (pesticides or polyaromatic compounds)
- physical properties (melting range, consistency)

To obtain oils with desirable properties, it is necessary to submit them to further processing. This processing includes: (1) refining and (2) modification.

1. Refining

Refining processes are used to purify oils from various impurities mostly present as minor components. Several of these components are listed below together with the problems they may cause:

Impurities to be removed	**Problems caused by impurities**
phosphatides	difficulties in processing
sugars and sugar esters	
metals	oxidation (pro-oxidators)
oxidised components	negative taste, bad smell
pesticides	safety
polycyclic aromatics	safety
colouring substances	colour should be almost absent
volatile components	negative taste, bad smell
fatty acids	negative taste in some cases
partial glycerides	effect on crystallization, frying properties

Most refining processes are rather old. Traditional oil refining consists of four steps:

1.1. Degumming

Aim of the treatment:

- removal of phosphatides, sugars, and metals.

Execution of the process:

- addition of water to make phosphatides insoluble
- separation of the lecithin thus formed by centrifugation
- further reduction of phosphatides by treatment with phosphoric or citric acid

Developments:

- Membrane filtration may be applied instead of centrifugation. However, the flux rate in this process is rather low making the process too expensive.
- The "superdegumming process" ends up with a very low phosphorus content by using citric acid and in some cases a degumming aid in a special procedure.

1.2. Neutralization

Aim of the treatment:

- Removal of free fatty acids, further partial removal of phosphatides, sugars, oxidised components and colouring substances

Execution of the process:

- addition of caustic or soda ash/silicate
- batch or continious operation

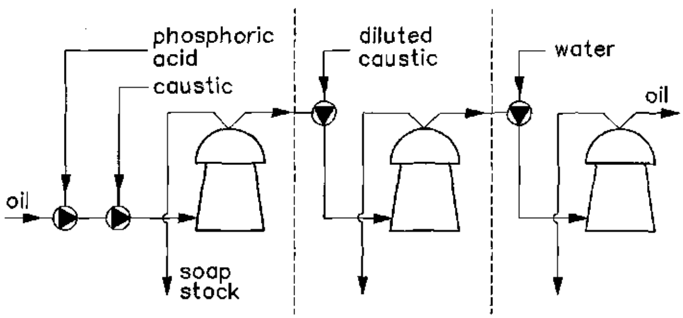

Figure 1. Continuous neutralization process.

Problems:

- high amounts of oil lost
- soap present in the effluent has to be splitted again

Developments:

- liquid-liquid extraction process with methanol applicable to oils with a very high fatty acid content (approx. 10 - 20 %)
- "physical refining" (see later)

An example of a continuous neutralization process is shown in figure 1.

1.3. Bleaching

Aim of the process:

- removal of colouring substances, oxidised compounds, metals, and polyaromatic hydrocarbons

Execution of the process:

- 80-110 C in the presence of 0.25-1.00 % diatomaceous earth; sometimes active carbon

Problems:

- oil losses (approx. 1% oil / 1% bleaching earth)
- disposal of spent bleaching earth

Solutions:

- use of synthetic earth (e.g. silica, alumina) effective at lower concentrations
- regenerating of the bleaching earth by a "wet oxidation process"

1.4. Deodorization process

Aim of the process:

- removal of volatile components, partial glycerides, fatty acids, colouring substances, and pesticides

Execution of the process:

- Steam destillation carried out at 0.3-1.0 kPa and 180-240 °C

During the last decade, the "physical refining process" has been developed and applied by many refineries. In this process the degumming is done more thoroughly and the neutralization step is omitted. The fatty acids are removed in the deodorisation step. Although this procedure has lower costs and better yields, it cannot be applied for all types of oils. Palm oil is the best known example for its successful application.

2. Modification processes

Modification processes are applied to provide the required components for products such as margarine, bakery fats and confectionary fats out of various crude oils with different compositions and prices. Modification changes the composition of the fatty acids and triglycerides and therefore the consumer properties. The triglyceride composition determines mainly the physical properties of the oil, like melting behaviour, crystallization rate, consistency. The fatty acids are the main components giving the nutritional value, taste and taste stability. Minor components also contribute to the nutritional value, taste and taste stability both in a positive (e.g. vitamins) and in a negative manner (e.g. metals, pesticides). A schematic overview of oil modifications is presented in figure 2, while oil modification techniques are summarized in figure 3.

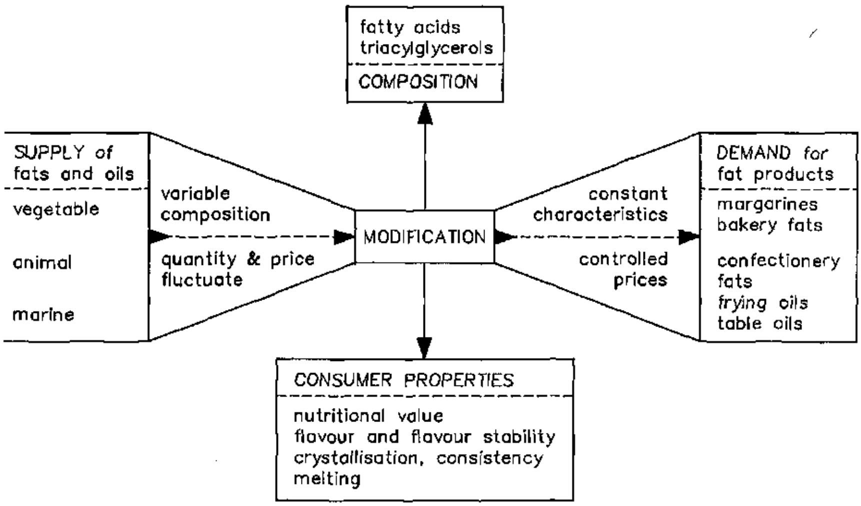

Figure 2. Oil modifications.

2.1. Fractionation

Because an oil or fat consists of many triglycerides with different melting properties a higher melting fraction and a lower melting fraction can be obtained by partial crystallization. The two fractions may be separated using several procedures. Dry fractionation is the most simple, batch technique where the crystals are seperated from the oil by filtration (e.g. drum filtration, membrane filtration or membrane filter press).

The yield of this procedure can be increased by washing the oil out of the fat crystals with a detergent solution. A very powerful fractionation is achieved by applying this technique in a solvent (most often acetone). This results in a much sharper separation.

An industrial example is the fractionation of palm oil. Figure 4 gives the melting curve for palm oil and fractions.

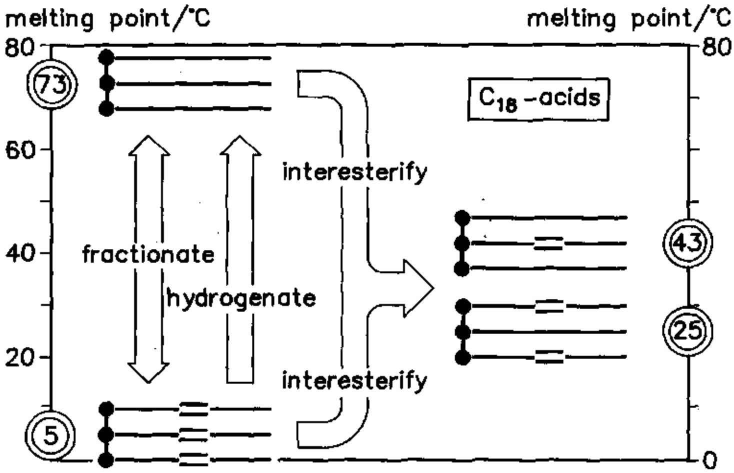

Figure 3. Oil modification techniques.

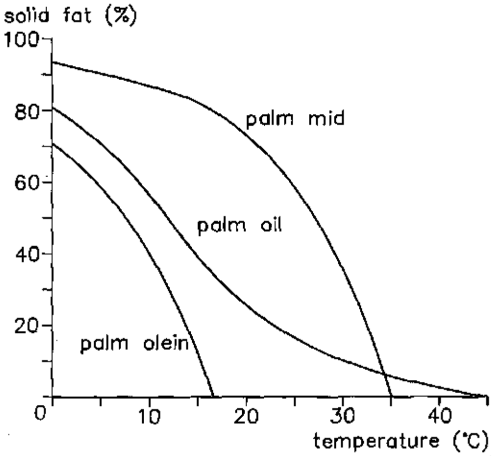

Figure 4. Melting curves for palm oil.

2.2. Interesterification

Interesterification is used to redistribute fatty acids over the triglycerides in order to influence the melting behaviour and the consistency. Depending on the starting materials the melting range is either raised or lowered as shown in figure 5. A new development in this field is the application of enzymes which - in contrast to the random interesterification products obtained by the traditional chemical procedure - result in very specific, new fats and oils; in many cases the enzymes are specific for the 1 and 3 position in the triacylglycerol.

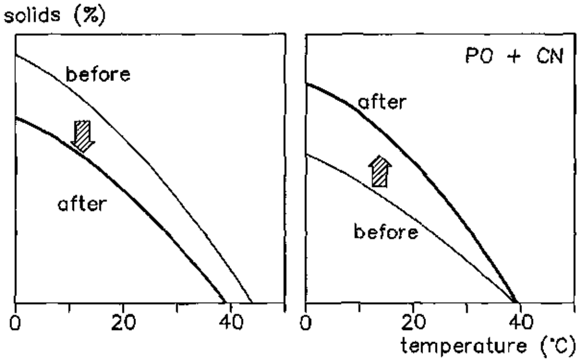

Figure 5. Effect of interesterification on the melting range.

2.3. Hydrogenation

Hydrogenation of triacylglycerols is often desired to make the fatty acid less unsaturated and thus lowering its melting point as well as its sensitivity to oxidation which causes an improvement in taste and taste stability. Hydrogenation is for example unavoidable in the case of fish oils in order to get rid off their fishy smell and taste.

The reaction is usually carried out in batch reactors in the presence of hydrogen and a solid catalyst (usually nickel) at a temperature between 100 - 200 °C. The process conditions stongly influence the oil quality. Therefore, process control is very important for getting the right end-conditions. A nice example for the need of process optimization is the hydrogenation of soyabean oil where the selective conversion of linolenic to linoleic acid is required but where any further hydrogenation would be disadvantageous. The main influencing factors are:

- the concentration of hydrogen in the bulk of the oil
- the quality and
- the pore size of the catalyst.

The selectivity of the process is determined mainly by the pore size of the catalyst what means that by choosing a catalyst with narrow pores, we get total hydrogenation whereas a catalyst with wide pores results in partial and selective hydrogenation. Various reactor types used for hydrogenation are schematically shown in figure 6.

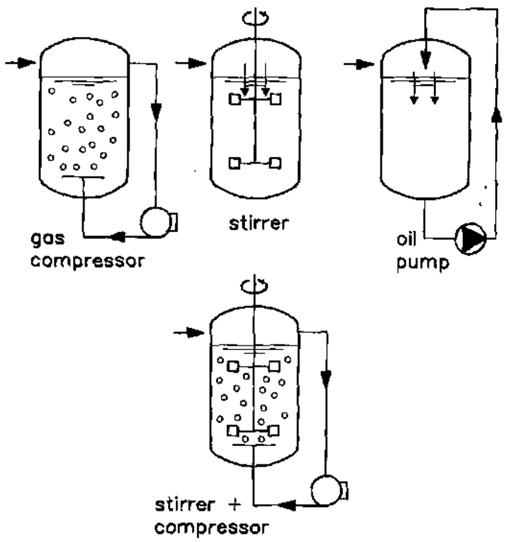

Figure 6. Reactor types used in hydrogenation.

In conclusion it may be stated that although refinary and modification processes have already been applied for about a hundred years, they still have a scope for improvements in certain directions.

SEPARATION PROCESSES OF FATTY ACIDS

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Fatty acids are produced by splitting different fats and oils, mostly triglycerides, whith glycerol as the other important product. Fatty acids and a great number of their derivatives - like esters, dimer acids, salts, fatty nitriles (used as fatty nitrogens), fatty acid methyl esters and fatty alcohols - have a broad industrial application. Because of their surface active properties, these fatty materials are mainly used as soaps and detergents and fatty nitrogen chemicals. But they are also important additives in paints, pharmaceuticals and lubricants. For these purposes different, special fatty acids are needed in a pure form. Specific polymers can be produced based on unsaturated fatty acids, whereas the absence of these compounds is desirable in many other application areas. In the food industry, certain fatty acids are reconverted to new fats and which then have the right composition, better processing qualities and good nutritional values.

The separation of fatty acids, especially the separation of unsaturated from saturated fatty acids, in an industrial process is often based on the differences in melting or boiling points. Therefore, a crystallization or distillation operation is commonly used. Usually, saturated fatty acids have higher melting point than their unsaturated homolog. By decreasing the temperature, the saturated fatty acids will crystallize first and can be separated from the unsaturated ones. In the case of distillation, particularly when highly unsaturated fatty acids are concerned, the high temperature may cause polymerization and other side reactions.

The following separation procedures are carried on an industrial scale:

1. Panning and pressing

Decreasing the temperature results in crystallization of the saturated fatty acids, while the unsaturated fatty acids remain in an oil form.

Consequently, the oil fraction (the unsaturated fatty acids) is forced out of the crystals. The main disadvantages of this method are that significant amounts of oil remain between the crystals, resulting in a partial separation of the fatty acid fractions and the large vessel volume required for this operation.

2. Hydrophilization process

Here also, a crystallization is the first step. By the addition of a surfactant solution, the unsaturated fatty acid phase is solubilized in the water phase, forming an emulsion. After phase separation, the unsaturated fraction is obtained. The crystalline fraction is heated to obtain the saturated fatty acid fraction in a separate oil phase.

3. Solvent crystallization

This method is also a well known industrial procedure in which the separation is achieved due to differences in solubility in a suitable solvent, however, the selectivity is not very high, resulting in rather impure products.

The advantages and disadvantages of these classical industrial processes are given in Table 1.

Table 1. Characteristics of the most important classical separation processes.

Hydrophilization	Crystallization	Distillation
*works on most FA *low operation costs *good olein quality	*works on most FA *highly automated *good stearin quality	*mainly saturated FA *good quality all FA*
*high investment costs for small capacities	*high investment costs *high operation costs	*difficult separation between homologs *high operation costs

New developments to improve selectivity include:

1. Membrane techniques
2. Use of specific reactions
3. Affinity processes

The combination of these three techniques may further enhance the yield and selectivity of separation processes. Some examples will be given to illustrate this.

1. <u>Integration of reaction and membrane separation.</u>

Lipases are capable to hydrolyze fats and oils under mild conditions. This can especially be an advantage in the case oils are used that are very susceptable for oxidation reactions. This process is based on the use of a lipase immobilized on the surface of a membrane. The reaction can be specific in the case selective lipases are used. The specificity of the enzyme may be oriented to the site of the hydrolysis (site specific lipases) or to the fatty acid (fatty acid specific lipases). For the application of these enzymes two routes can be envisaged. Firstly, using an aspecific lipase the oil can be hydrolized completely. This requires a fat/fatty acid separation and consequently a separation between the fatty acids. Secondly, the use of specific lipases results in a partial hydrolysis, and only the fatty acids formed have to be removed from the oil, resulting in the fatty acids required. In this route no further separation is required. The prices of these enzymes are of course quite high, making an effective reuse of the enzyme necessary. This can easily be achieved in a hollow fiber membrane reactor, as has been developed in our department. The membrane device also provides an effective phase separation.

The membranes used are hydrophilic, and are therefore permeable for water and glycerol but not for the glycerides and fatty acids. The diameter of the fibers and the wall thickness is approximately 200 m and 6 m, respectively. The lipase is immobilised on the inner surface of the fibers. The oil flow is inside the fibers and the water phase is circulated outside. A schematic view of a continuous countercurrent flow reactor is given in figure 1. The half life time of the enzyme in this type of reactor appears to be more than 1000 hours, which ensures a rather effective reuse of the enzyme.

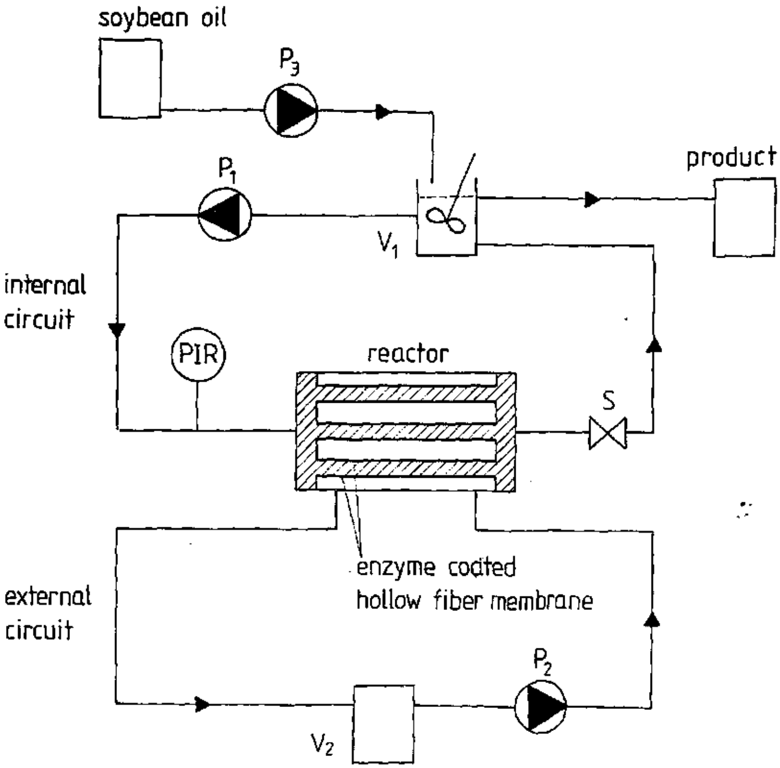

Figure 1. Immobilized lipase hollow fiber membrane bioreactor for the hydrolysis of oil.

It appears that the hydrolysis rate strongly decreases as the concentration of free fatty acids (FFA) increases. For this reason, the continuous removal of the FFA from the oil is necessary to maintain a sufficiently high reaction rate. Conventionally, sodium hydroxide is added to the oil to saponify the fatty acids. Consequently the thus obtained soapstock can be removed by centrifugation. The major drawback of this method is the inclusion of triglycerides into the soapstock. The amount included usually equals the amount of fatty acids removed and have to be considered as a loss. For this purpose a membrane process has been developed in our department, not having the inclusion problem. The addition of 2-propanol together with the usual sodium hydroxide solution to the FFA rich oil (FFA content 10-20 %) results in the formation of a dispersion. Consequently this dispersion is separated by two membranes in series. The hydrophilic part of the dispersion (water, 2-propanol and the soaps) permeates through the hydrophilic membrane. The hydrophobic oil is capable to permeate through the second, hydrophobic, membrane. In the permeate of the hydrophilic membrane no triglycerides and in the permeate of the hydrophobic membrane no fatty acids can be detected, indicating that the separation is complete. If a specific lipase is used, the desired fatty acid can then be obtained directly from the water phase. The remaining oil can be recirculated to the enzymatic fat splitter. This process is schematically depicted in figure 2.

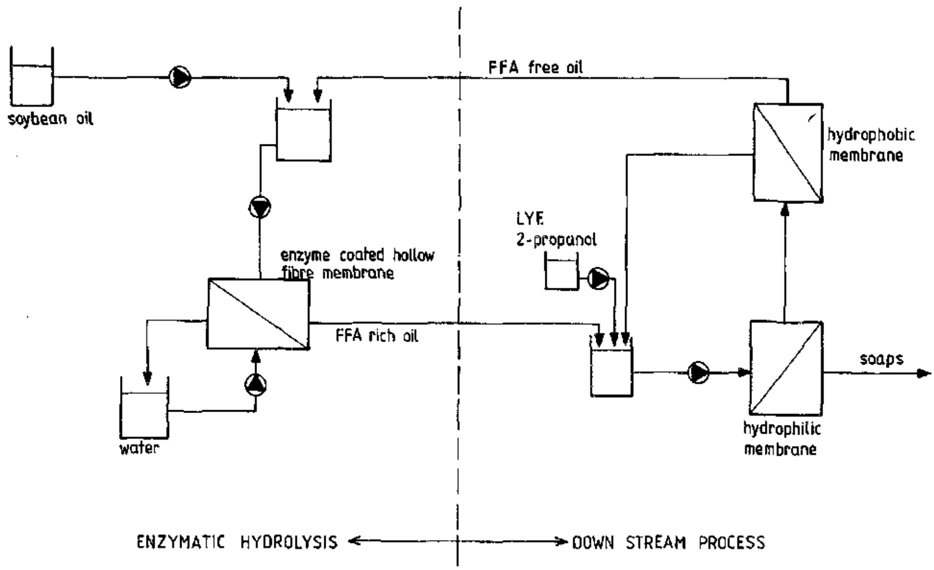

Figure 2. Combined enzymatic hydrolysis and downstream process.

A number of commercially available membranes, both hydrophilic and hydrophobic, have been tested for their capability to separate this dispersion. From the data given in Table 2, it is clear that the combination of the hydrophilic polyacrylonitrile membrane with the hydrophobic polysulfone or PVDF membrane will give a good separation for FFA oil mixtures.

Table 2. Properties of several commercial membranes.

Type of membrane	cut off	flux [$l/m^2.h.bar$]
hydrophilic membranes		
polyacrylonitrile (PAN)	30,000	30
Cellulose	10,000	3
Cellulose-acetate	200,000	*
Polyamide	0.2 m	*
hydrophobic membranes		
Polysulfone	30,000	5
Polypropylene	0.2 m	*
PVDF	0.14 m	*
PVDF	0.2 m	16(Pmax=0.3 bar)
PVDF	10,000	*
PTFE	0.2 m	*

* Permeation of both phases

2. Affinity separations.

Specific binding of a desired compound to an adsorbent is the basis for affinity separations. Unsaturated fatty acids are capable to form a - bond with silver ions. If the silver ions are connected to an inert carrier like silica, the unsaturated fatty acids can be separated from a fatty acid mixture. A selectivity of about 10 is obtained as shown in figure 3. The application of silica on a large scale is difficult, however, this technique can be useful for the separation of small amounts of unsaturated fatty acids from a mixture.

It is also possible to remove saturated fatty acids from the mixture. For this purpose, cyclodextrins can be used. Cyclodextrins are cyclic water-soluble oligosaccharides with an apolar cavity. Alfa-cyclodextrins can form inclusion products with saturated fatty acids, whereas the unsaturated fatty acids are not included. Here also, the combination

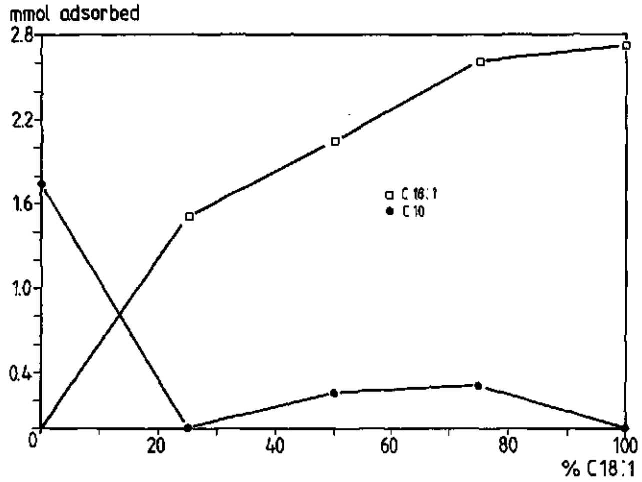

Figure 3. Adsorption of fatty acid mixtures onto silver/silica in the presence of acetic acid to avoid aspecific binding.

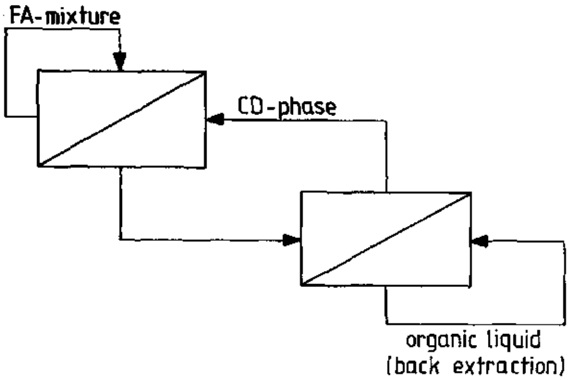

Figure 4. System for the removal of saturated fatty acids from a mixture by membrane extraction using cyclodextrins

with a membrane is very useful. The unsaturated fatty acids are released from the cyclodextrin complex by a back extraction with an appropriate organic solvent. In the system as depicted in figure 4 it is possible to recirculate the cyclodextrins, whereas no phase separations are required, since the membrane keeps the two phases separated. These results show that selective and mild processes may be developed to obtain fatty acids in a pure form both for the chemical industry and for the food industry. The application on an industrial scale, however, will take some time but will offer interesting opportunities in the future.

OLEOCHEMICALS VERSUS PETROCHEMICALS IN THE CHEMICAL INDUSTRY

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Until the middle of this century, natural oils and fats have been used extensively for the manufacturing of different products in various industries. Since that time, this situation has changed considerably in favour of petrochemicals because of the low cost of raw materials and due to the enormous investments in research and process development for this industry. The future situation might change as the oleochemical industry is trying to regain some shares in special market niches. In this presentation, basic characteristics and future prospects of the oleochemical industry will be compared.

1. RAW MATERIALS

Raw materials for both branches originate from biomass produced by photosynthesis. The difference is, that mineral oils were created millions of years ago, whereas oils and fats from plants are annually renewable resources (Fig. 1).

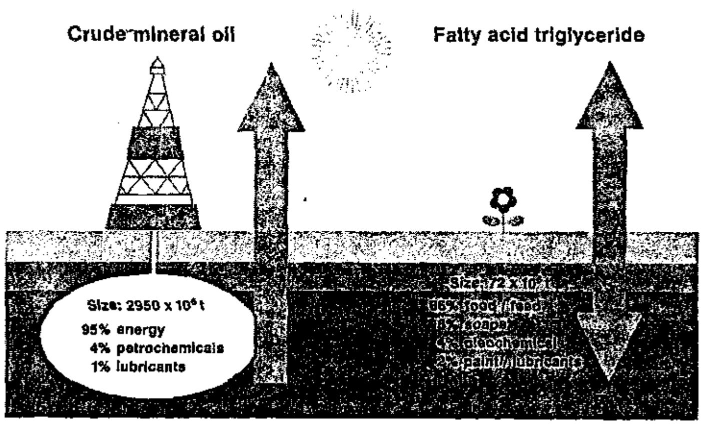

Figure 1. Size and application of the world oil market (1987).

The worldwide production of crude mineral oils is approximately 3 milliard tons/years, which is used for the following purposes:

- 95% for energy, causing a lot of environmental problems by increasing the carbon dioxide content of the atmosphere
- 4% for petrochemicals
- 1% as lubricants

The total production has been more or less constant over the last years. Volume for oils and fats (triglycerides) is 72 million tons, which is approximately 2.5% of the crude mineral oil tonnage. The production of triglycerides has strongly increased over the years due to large investments in for instance palm oil plantations. Triglycerides are used for the following purposes:

- 86% for food and feed
- 8% for the production of soaps
- 4% for oleochemichals
- 2% for paints and lubricants

2. BASIC PROCESSES

The petrochemical industry starts with oil fractions which are converted to smaller molecules in huge crackers. The basic intermediate products are olefins, especially ethene, propene and aromatics. These intermediates are sold in the market, for example aromatics as octane boosters in unleaded fuels. Most intermediates are, however, chemically converted into polymer products.

The structure of the petrochemical industry is based on very large multinational companies which are integrated backwards and forwards to other industrial branches (from exploration of crude mineral oil or production of consumers goods). At the same time there is also a tendency to develop national policies and to protect own energy sources.

The oleochemical industry is completely different, being much smaller in size and with less complicated processes. Although some multinational companies as for instance Unilever or Henkel have a considerable share in this market, smaller companies with production capacities below 30.000 tons/year exist. The basic processes are also quite different from the petrochemical industry.

The initial step is the splitting of the esterbonds in the triglycerides, resulting in glycerol and -depending on the splitting agent used- fatty acids, soaps or methyl esters. (Fig. 2).

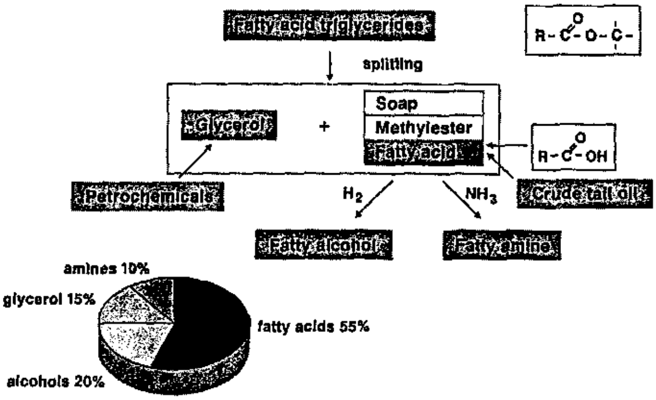

Figure 2. Basic oleochemical reactions

Most commercial reactions with fatty acids take place at the carboxylic group. This is remarkable if we consider that more than 80% of the mainly used C18 fatty acids available in nature are unsaturated. Most of the reactions performed are:

- fatty acids + H2 ⟶ fatty alcohols
- fatty acid + ammonia ⟶ fatty amides, fatty nitriles
- fatty nitriles + H2 ⟶ fatty amines

The main oleochemical products are fatty acids (55%), fatty alcohols (20%), fatty amines (10%) and glycerol (15%).

3. <u>OLEOCHEMICAL CHARACTERISTICS</u>

The oleochemical industry has certain advantages compared to the petrochemical industry.

a) <u>Raw materials</u>

- Renewable sources
- Growing market
- Relatively low priced
- Constant composition
- Interchangeability between resources

b) Processes

- Environmental friendly
- Medium investment costs
- Mature for standard products

c) Endproducts

- Nontoxic
- Biodegradable
- Linear, clearly defined chainlength (C8 - C22)
- Clearly defined position of functional groups

4. FUTURE POSSIBILITIES FOR OLEOCHEMICALS

In addition to the advantages given above, new developments will offer good possibilities for future growth. The exploitation of new results of biotechnology brings tremendous new opportunities. In the field of raw materials good results have already been achieved by plant breeding, resulting in a higher content of certain fatty acids as for instance high oleic sunflower, better harvesting properties, resistance against pesticides, and less climate dependency. With regards to oleochemical processes, possibilities are open for process optimization, computerization, and the use of special techniques as for example reactions under influence of ultrasonic waves. The introduction and further developments of new biochemical reaction processes are also very promising.

Such reactions have several advantages over the usual chemical routes, including better selectivity, less by-products resulting in higher yields, milder conditions, less energy demand, and possibly new products. The exploitation of different reactions at the double bound will open completely new opportunities.

Much work has already been started in many of these fields, even on industrial scale. One example is the enzymatic esterification of fatty acids with alcohols at low temperatures resulting in esters with colour and odours unmatched by standard chemical reactions. These products are presently distributed in the cosmetic industry but other applications will follow soon.

5. ATTRACTIVE END-MARKETS FOR OLEOCHEMICALS

Oleochemicals have certain advantages against petrochemicals in a number of applications:

a) Food and Feed

More and more food is processed on large industrial scale. Emulsifiers and many other additives are used in these processes and products based on natural raw materials have obvious advantages.

b) Cosmetics

A clear move is seen in the direction of 'back to nature' in which two main tendencies may be observed. Oleochemical raw materials are preferred against petrochemicals and products based on vegetable oil are preferred over animal origin. A new development of the oleochemical industry, esters of branched fatty acids and alcohols, which resemble the skin grease of waterbirds, are going to have interesting applications. These compounds form a film on the skin which -in contrast to the fatlayer formed by the usual lotions- is permeable for water, vapour and air. This opens the realisation of completely new concepts in cosmetics.

c) Surface active reagents

'Away from petrochemicals' - that is the tendency in this field. The main reason is environmental protection and biodegradability. The biodegradability of the detergents based on aromatic petrochemicals causes problems, products based on oleochemicals are much better in this respect. They offer a great diversity to fulfil various application demands. Sulphonated alcohols, a-sulphomethylester, soaps and quarternary ammonium salts are the most important groups of products.

d) Lubricants

Parallel to technical developments, the demand for lubricants has shown quite big changes. In earlier times natural oils were used, later, with the development of different engines, lubricants based on mineral oils got strong preference. Today, the two main aspects in choosing the right type of lubricants are energy improvement and biodegradability. Fatty acid esters are becoming more important now since they are completely biodegradable and allow both much higher and lower operation temperatures compared to mineral oils. These low operating temperatures, combined with a much better viscosity temperature relationship, reduce the energy consumptions and allow much higher operation temperatures compared to mineral oils. These high operating temperatures reduce the energy consumptions.

The size of the European lubricant market is 5.5 million tons p.a. As the market share for the fatty acid esters is presently only 0.2%, lubrication is one of the highest growth potentials for oleochemicals.

e) <u>Plastics</u>
A large part of the fast growing 'Engineering Plastics' consist of traditional polycondensates like polyamides, polyesters and polyurethanes. Properties such as flexibility and hydrolytic stability of these products are drastically improved by replacing the mainly used C6 dicarboxylic acid (adipic acid) by longer chain dicarboxylic acids that are mainly produced from oleochemical raw materials. (Fig. 3).

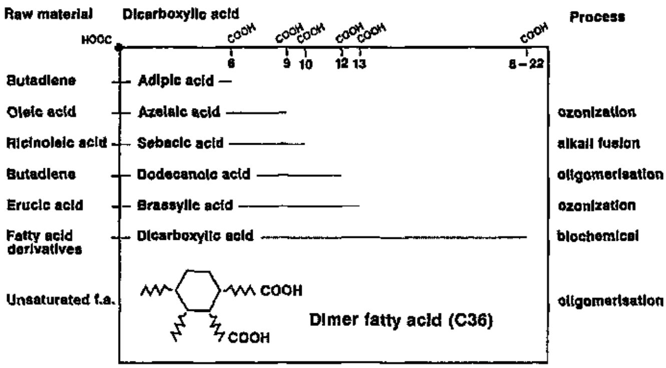

Figure 3. Important dicarboxylic acids for polycondensates.

The longest chain dicarboxylic acids commercially available, which also exhibit the highest purity are the C36 and C44 discarboxylic acids (dimer acids). These acids allow a new dimension in the design of modern engineering plastics. In addition to these available C36 and C44 dicarboxylic acids several new biochemical routes for dicarboxylic acids are under study.

INTRODUCTION AND BREEDING OF NEW OIL CROPS

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1. Introduction

Vegetable oils of only ten crop plants are presently utilized in a variety of industrial products. Important non-food oil-seeds are linseed, castor, tung, soybean, rape seed, coconut and palm kernel. However, there is an increasing interest worldwide in novel oil-seed crops which can produce specific fatty acids such as unique long-chain, hydroxy, conjugated and epoxy fatty acids. Some of these renewable oils can be alternatives for petrochemicals, for the production of lubricants, plastics, surfactants and polymers. Various novel oil-seed crops, producing these unusual fatty acids have been introduced and evaluated since 1986 at Wageningen.

2. Objectives and economic benefits of new oil crops

The objectives and economic benefits of new oil crops can be divided into three major categories.

Agricultural aspects:

- broadening the present narrow crop rotation;
  Especially in the Netherlands, there is a real need for an increase in the number of crop types. The present way of agriculture cannot be continued because of the intensive utilization of agrochemicals.
- providing alternative non food crops for the present crops with overproduction.

Industrial aspects:

- oleochemicals may compete with petrochemicals, they can have better performance in some applications;
- novel oils of different chemical structure will allow development of new and useful products;
- domestically produced oils would both increase stability of supply, quality and price of the industrial feedstock.

General aspects:

- Substitution of fossil fuel reserves used for petrochemical products by renewable oleochemical feedstock means a less rapid depletion of these reserves (environmental benefit);
- oleochemicals are generally known for their good biodegradability (e.g. lubricants);
- renewable sources "domestically" produced will reduce imports in the European Economical Market;
- new employment opportunities may occur particularly in the agrotechnical sector.

3. Introduction of new oil crops

Between 1986-1989 more than 40 different novel oil crops have been introduced in Wageningen. In some cases several species of one genus, e.g. Cuphea, Crepis, Dimorphotheca, Limnanthes and Oenothera have been introduced. First introduction of a crop starts always on a limited scale. Only a few accessions of one particular crop are introduced, grown in single plots of 3-10 m^2, depending on the amount of seed available and preliminary evaluated. Both an agricultural and chemical evaluation is conducted. For the agricultural evaluation a standard descriptor-list including important properties is used. Oil content and fatty acid analyses are conducted by the ATO, Wageningen. The introduced material originates mainly from botanical gardens and gene banks all over the world. After an initial evaluation of one or two years a selection of the most promising crops based on agricultural and chemical performance is made. To assess the chemical feasibility of the novel oil crops close cooperation has been set up with a number of industries. The gene pool of the most promising crops will be broadened according to the availability of the material. Table 1 presents a number of crops introduced up to 1989.

4. Selected oil crops of interest in the near future

4.1. General characteristics

"Crambe" (Crambe Abyssinica)

General aspects:

Gene centre: Mediterrean, Ethiopia, Central Asia

Table 1. Novel oil crops introduced in Wageningen between 1986 - 1989

type of fatty acid	crop species
short and medium chain length (C8 - C14)	*Cuphea* spp.[3]
C16 - C18 fatty acids	*Euphorbia lathyris*[3]
- C18:1	*Camelina sativa*[3]
- C18:3	*Lallemantia iberica*[3]
- C18:1/C18:2	*Helianthus annuus*[2]
long chain fatty acids (C20 - C24)	
- particularly erucic acid	*Crambe abessinica*[1]
	Eruca vesicaria[2]
	Lunaria annua[2]
- unique fatty acids	*Limnanthes* spp.[1]
hydroxy fatty acids	*Dimorphotheca* spp.[1]
	Lesquerella spp.[2]
	Cardamine impatiens[3]
	Osteospermum spp.[2]
epoxy fatty acids	*Crepis biennis*[3]
	Euphorbia lagascae[2]
	Vernonia spp.[3]
conjugated fatty acids	*Calendula* spp.[2]
	Osteospermum spp.[2]
other unique fatty acids	
- petroselenic acid	*Coriandrum sativum*[2]
	Foeniculum vulgare[2]
- gamma-linolenic acid	*Oenothera* spp.[2]
	Borago officinalis[3]
- crepenynic acid	*Crepis alpina*[2]

1. promising novel oil crops, further introduction will be conducted
2. crops still under consideration, further introduction planned
3. crops dropped from the introduction programme

Gene pool: limited (100 - 150 nrs.)

some varities and wild forms

Alternatives: rape seed (specially "high erucic"),

Eruca vesicaria, mustards

Agronomical aspects:

yields: 1000 - 2000 kg seed/ha

cultivation: clay/sandy soils form april to august, winter cultivation not yet feasible

rotation: susceptible beet cyst nematode (BCN)

Chemical composition:

oil content:	28 - 45 %	(35 %)
C22:1 (erucic acid):	47 - 62 %	(60 %)
protein:	17 - 35 %	

glucosinolates present

Applications:

erucamides (polyethylene films)

lubricants (esters)

printing inks

meal as byproduct (protein)

Limitations:

yields too low, therefore competition with rape seed (yields above 2800 kg/ha)

low prices of erucic acid

glucosinolate content (4 %) for utilization in meal

Expectations:

The increasing demands of industrial applications of erucic acid, the tendency to grow more 'double zero' rape leaves good possibilities for alternative erucic acid crops.

African daisy (*Dimorphotheca pluvialis*)

General aspects:

Gene centre: South Africa, Angola

Gene pool: small (50 - 100 nrs.)

Alternatives: *Ricinus communis*

Agronomical aspects:

yields: 500 - 1500 kg seed/ha

cultivation: clay/sandy soils form april to august,
rotation: very susceptible to M. hapla

Chemical composition:

oil content:	15 - 22 %
dimorphecolic acid:	25 - 60 %
protein:	20 - 35 %

Applications:

surface coatings
plastic foam
lubricants
surfactants

Limitations:

low yields (irregular seed ripening and poor seed retenti on, harvest/cleaning techniques) influence of climate on oil content, in general low in N.W. Europe
industrial applications still not exactly known

Expectations:

Dimorphecolic acid imparts chemical features of tung and castor oils.
Technical-chemical applications in medium short future by seed yields above 2500 kg/ha.

Meadowfoam (Limnanthes alba)

General aspects:

Gene centre: N.W.America
Gene pool: very small (10 - 20 nrs.)
limited genetic diversity
Alternatives: L. douglasii

Agronomical aspects:

yields: 500 - 1500 kg seed/ha
cultivation: clay/sandy soils
winter
rotation: partly susceptible beet cyst nematode (BCN)
hostplant M. hapla
short cycle (e.g.-100 days)

Chemical composition:

oil content: 20 - 30 %

unique long chain fatty acids:		95 %
C20:1ω	15	53 - 64 %
C22:1ω	17	9 - 20 %
C22:2ω	9, 17ω	19 - 31 %

Applications:

- lubricants
- cosmetics
- plasticizers
- meal (20 - 25 % protein)
- paints (drying problems)

Limitations:

- low yields (poor seedset, seed retention)
- low oil content
- glucosinolate content (4 %) for utilization in meal

Expectations:

Oleochemical and lubricant industry are interested.

Oil price determine feasibility.

Perhaps biodegradable products and cosmetics may bring about a break-through.

4.2. Economic aspects

The average realized yields of the three selected crops together with their estimated potential yields after 5-10 years of breeding and crop improvement are given in Table 2. A cost calculation for the three selected novel oil crops is presented in Table 3. These calculations in Dutch guilders are based on the following assumption for the 2000:

Production cost of farmer per ha	900
Nett return farmer per ha	800
Cost of cleaning and drying / ton	200
Cost of oil extraction and refining / ton	400
Seed meal benefit / ton	350

The estimated cost to produce these oils without EEC subsidy is at present too high to consider commercial cultivation in The Netherlands. However, the cost of Limnanthes oil without EEC subsidy is in the range of the price of the Oregon Meadowfoam Growers Association, U.S.A. and far lower than the price of Jojoba oil. Both oils are therefore mainly

Table 2. Realized and potential yields of novel oil crops.

Species (main fatty acid)	% oil a	% oil b	seed yield [kg/ha] a	seed yield [kg/ha] b	oil yield [kg/ha] a	oil yield [kg/ha] b
<u>Crambe abyssinica</u> (60 % erucic acid)	35	42	2000	3000	700	1260
<u>Dimorphotheca pluvialis</u> (60% dimorph. acid)	22	27	1500	2500	330	675
<u>Limnanthes alba</u> (90-95% unique long chain fatty acids)	23	28	800	1500	184	420

a: average realized yields
b: potential yields

Table 3. Cost calculations of novel oil crops.

Species (main FA)	oil yield [kg/ha] a	oil yield [kg/ha] b	estimated cost (Dfl.) of oil per litre I a	I b	II a	II b	III a	III b
<u>C. abyssinica</u> (60% erucic acid)	700	1260	3.50	2.30	2.45	1.70	1.24	0.56
<u>D. pluvialis</u> (60% dimorph.acid)	330	675	6.60	3.80	4.40	2.70	3.10	2.50
<u>L. alba</u> (90-95% unique long chain FA)	184	420	11.0	5.30	6.60	3.60	8.80	1.65

I : no subsidy
II : with EEC subsidy of Dfl. 750 / ha
III : With EEC subsidy of Dfl. 580 / ton (as 'double zero' rape)
a : average realized yields
b : potential yields

utilized in cosmetics. The costs of the oils are becoming more realistic for industrial utilization when the present EEC subsidies will be applied. Realization of potential yields together with EEC subsidy would make a commercial cultivation of these oils for certain industrial applications feasible. It is encouraging that the EEC is at present considering to subsidize high erucic rape seed in the same way as 'double-zero' rape seed.

5. Breeding of new oil crops

To improve the oil yields per ha, to achieve optimal oil compositions and thus increasing the marketing possibilities, the following aspects with respect to breeding activities have to be considered.

5.1. Genetic aspects
- extention of gene pool and
- genetic improvement of the crops in the direction of new and better varieties

5.2. Selection criteria to obtain crops with improved yields
- "winterhard" types (e.g. L. alba)
- synchronization of flowering and subsequent seed ripening (e.g. Dimorphoteca)
- improved seed retention (all crops)
- design of ideal crop ideotypes
  - increased harvest index of all crops. The harvest index of the novel oil crops are much lower than those of cereals and some existing oil crops
  - selection for vigorous plants (e.g. L. alba)
- hybrid vigour, heterosis by crossing gene pools of different origine (e.g. Crambe abyssinica)
- higher oil content

5.3. Oil composition
- changes into desired fatty acid composition
- searching for unique types of fatty acids

Some unique types of fatty acids that have been detected are given in figure 4.

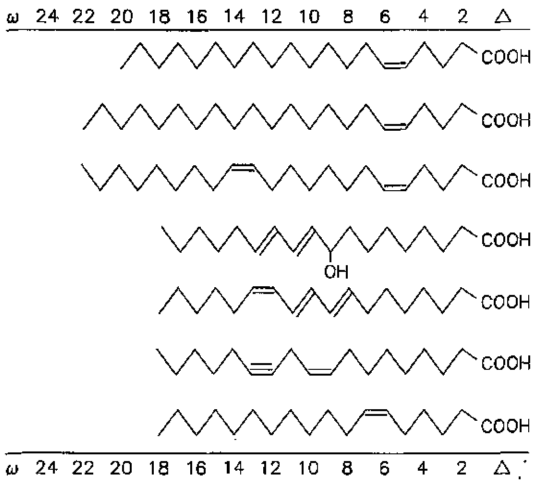

Figure 1. Unique fatty acids in oleaginous plants.

6. Conclusions

- The introduction (e.g. broading gene pools) and preliminary evaluation of possible novel oil-seed crops containing fatty acids with molecular variations should be continued;
- limited quantities of these oils should be distributed to oleochemical and final processing industries in order to encourage industrial evaluation and possible application;
- a number of selected oil-seed crops need further crop and genetic improvement with the aim to increase oil yields/ha;
- presently, some selected novel oil-seed crops can only be considered for commercial cultivation when they will be subsidized according to rape seed regulations of the EEC.

PLANT BREEDING FOR THE IMPROVEMENT OF FATTY ACID COMPOSITION OF SEEDOILS

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Several general principles have to be considered when breeding for the improvement of fatty acid composition of seed oils. It is important to realize that a strict correlation exists between the yield of a particular oil crop and the price of the oil produced from such crop. With only slight variations the lowest oil prices are associated with the highest yielding crops. Rapeseed and sunflower for example belong to this latter category whereas for instance mustard yields a more expensive oil. This correlation holds for whatever purpose the seed oils are used and is also independent on whether the oil is used as such or as a specific oil. Specific oils will also have higher prices whenever the yield of the crop is lower.

Another very important aspect is the yield potential of various crops. Yield vary considerably between the different species ranging from 500 to 3000 kg/ha or more. But the questions is to what extent this yield is a specific characteristic of a species and how much it depends on the input already given to the crop. For instance the yield of rapeseed has increased considerably over the last 30 years. In general, however, oil crops have not had such a long history of breeding as for instance wheat which has been planted since over 2000 years. This also means that cultivation techniques for oil crops are less well developed than those for wheat. Consequently, it depends on what level of genetic and agronomic improvement two species are compared and connected with that, what input is necessary for one species to arrive at an equal level with the other species.

For industrial oils the quality requirement is of course another very important aspect and it differs very much from those for food oils. The latter - produced by traditional oil crops - have a rather uniform molecular composition consisting of fatty acids of mainly 18 carbon atoms and one or two double bonds at the $\Delta 9$ and $\Delta 12$ positions (Table 1).

Table 1. Fatty acid composition (%) of some traditional oil crops.

Fatty acid		Palm kernel	Palm	Olive	Sun-flower	Soja	Rape-seed	Zero rape
Lauric	12:0	54						
Palmitic	16:0	8	44	10	7	11	3	4
Stearic	18:0	2	5	2	3	3	1	1
Others		16					2	2
Total saturated		80	49	12	10	14	6	7
Oleic	18:1	17	40	80	30	26	12	60
Linoleic	18:2	3	11	8	60	53	13	21
Linolenic	18:3					7	9	11
Erusic	22:1						50	1
Others							10	
Total unsaturated		20	51	88	90	86	94	93

Industrial utilization of fatty acids and derivatives (Table 2) needs a much broader diversity. It requires unusual, specific fatty acid compositions as well as the presence of the target fatty acid in a high percentage in total. Requisites for making an oil unusual are firstly variation in chain length and secondly variation in functional groups.

Table 2. Industrial use of fatty acids with varying chain lengths.

Long chains (> C16)	Medium chains (C8-C16)	Short chains (< C8)
coatings	surfactants	plasticizers
plastic additives	detergents	alkyds
stabilizers	emulsifiers	lubricants
plasticizers	soaps	
fabric softeners		
adhesives		

An idea about the variation in chain length required may be obtained by a comparison of the world supply of fats on the one hand and the demand by a large European producer of fat derivatives on the other hand (Table 3). Fatty acids with varying chain lengths occur in various plants and even within one genus the fatty acid composition may be quite different as shown for Cuphea seed lipids (Table 4). Variation in structure and position of functional groups is also available in nature. Examples of such unusual fatty acids with special functional groups found in some annual plant species are summarized in figure 1.

Table 3. Supply and demand of fatty acids.

Chain length	Supply [%]	Demand [%]
> C 18	5.9	0.8
C 18	68.4	37.9
C 16	17.5	17.0
C 14	3.2	10.8
C 12	3.7	25.3
< C 12	1.3	8.2

Table 4. Variations in fatty acid composition (%) in Cuphea.

Species	Fatty acid						
	8:0	10:0	12:0	14:0	16:0	18:1	18:2
C. racemosa	-	-	0.1	0.2	15.3	17.4	58.8
C. palustris	19.7	1.4	2.0	63.7	6.7	3.0	2.9
C. tolucana	-	23.0	63.3	4.5	1.8	1.9	5.0
C. paucipetala	1.2	87.4	2.0	0.8	1.9	1.8	4.0
C. painteri	65.0	24.0	0.2	0.4	2.8	3.3	3.9

Elaeostearic acid
Centranthus macrosiphon

Calendic acid
Calendula officinalis

Parinaric acid
Impatiens balsamina

Dimorphecolic acid
Dimorphoteca pluvialis

Densipolic acid
Lesquerella lescurii

Lesquerolic acid
Lesquerella gracilis

Vernolic acid
Euphorbia lagascae

Crepenynic acid
Crepsis alpina

Petroselenic acid
Coriandrum sativum

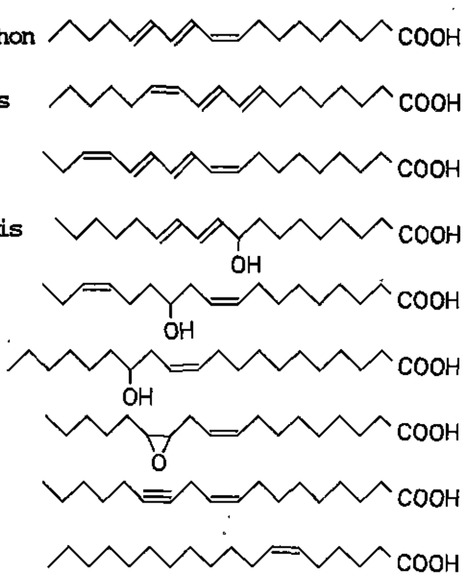

Figure 1. Unusual fatty acids in seed oils of annual plant species.

In respect to breeding demands three categories of plants may be distinguished:

1) a wild plant species with high quality oil which requires, however, a complete domestication program
2) intermediate situation consisting of a plant species with the desired seedoil quality which has so far been cultivated for other reasons only, and
3) a high performing oil crop species lacking the desired oil quality which has to be introduced.

One example will be given for each of these three categories to illustrate the necessary work.

1. <u>Domestication of a wild plant species</u>

Large screening programs for new industrial oils led to the discovery of high qualities of special fatty acids in seeds of some wild plant species. The genus Cuphea for example - including a large number of annual herbaceous species - shows a broad capacity of fatty acid synthesis resulting in a wide array of fatty acids with the desired medium chain length (Table 4). However, all the available Cuphea species exhibit wild plant characteristics severely impeding agricultural production, such as seed dormancy, slow and non-uniform growth of the seedlings, sticky glandular hairs on stems and flowers, a continuous flowering and unequal ripening, and in particular early seed shattering.

Breeding programs were started with the aim to identify useful genetic variations and to develop genotypes better adaptable to agricultural production. Mutation experiments have been effective in promoting the domestication program and several useful mutants have been obtained with for instance non-sticky hairiness or improved growth habit. But other desired mutations for e.g. fruit shedding have not yet been discovered within the selected productive Cuphea species. This problem of early seed dispersal may however be coped with using alternatively technical solutions. Meanwhile a vacuum picking machine for multiple harvest has been developed.

2. <u>Adaptation of other cultivated species</u>
Several species which have so far been cultivated for other than seedoil uses have been found to contain unusual fatty acids and thus, have good perspectives for the production of specific oils. These species have undergone earlier domestication, but a "second cycle of breeding" is still necessary to achieve economic oil yields from them.

A first example is Calendula officinalis which has been cultivated as a medicinal plant used in osteoporosis; it is also well known by hobby gardeners as a nice ornamental plant.

A second example is Coriandrum sativum the seeds of which are produced for spice uses. Both these plants have a dominating fatty acid of unusual chemical nature and therefore may be very useful. In Göttingen Coriander was successfully grown on a 5 ha scale although in one year some problems were met with a bacterial disease, the socalled Coriander wilt. These two species are sufficiently preadapted to cultivation and ready to go as "industry oil crops".

3. Changing qualities of a high performing crop plant

Finally, the high agronomic performance of a traditional oilseed species can well be exploited in principle. Here, just a single mutation may be needed to change the biosynthetic pathway into the desired direction. For instance in Cuphea, the ancient taxa exhibit the normal C18 fatty acids in their seedoils, while the taxonomically more derived types synthesize shorter fatty acids only. This indicates that mutations may have occurred stepwise, inhibiting the elongation of the fatty acids. In a similar way, mutations are known in rapeseed blocking the erucic acid synthesis.

Other prominent examples of oil quality improvement by simple mutation are high oleic sunflower and high linoleic linseed. Evidently there is a good chance to find single genes in high performing crop plants responsible for an important improvement of and to oil quality. It is a common experience though that mutants frequently yield less than the original parents and that due to pleiotropic effects or essential physiological correlations disorders may occur, which necessitate an intensive genetic recombination work in appropriate plant breeding programs.

Because of the genetic difficulties and of the many years that the finalization of a new better cultivar takes, plant breeders have always been motivated to apply recent scientific progress at the earliest possible date. No wonder that modern biotechnologies and gene technologies have also attracted active interests to obtain results in a shorter time and by a more directed approach than with traditional plant breeding procedures. Already now plant breeding programs are benefiting greatly from modern biotechnologies. This is very true for instance for using in vitro culture methods. But one can also imagine, to identify a relevant gene and to transfer it into an interesting recipient crop. Especially rapeseed is a very good material because you can obtain protoplasts for somatic cell fusions and fusion products can be regenerated to plants. Asymmetric fusions are also possible and haploids can be made from microspores. High numbers of haploid embroids can hopefully be induced to deposit their fatty acids already at this stage allowing selections of the wanted gene at a very early stage. With a native gene of rape it has been demonstrated already that it can be inserted back into the plant where it becomes active again. In short, there is any reason to expect that molecular transfer of identified genes will soon be effective in rapeseed. But still a great many problems will remain to be solved which undoubtedly are crucial in this context. The oil storage process in particular is a very complex process and little is known on the conditions of cellular compartimentation.

MICROBIAL LIPIDS: PROSPECTS FOR BIOTECHNOLOGY

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Microbial technology as it is understood today principally deals with high value products which are produced in small amounts. However, for oils and fats we are dealing with, for the most part, a large volume market with concomitant low prices. What has then to be considered therefore is whether microorganisms can meet and answer this challenge. Although millions of tons of oils and fats are available from plants, microbial lipids may have certain advantages in special areas. Novel lipids not available from plants or animals are interesting. Polyhydroxybutyrate, a biodegradable polymer produced by bacteria illustrates this point to a certain extent. Although it is not a fatty acid-containing lipid it is nevertheless classified as a lipid in view of its solubility in chloroform and similar solvents. Another more clear example of a true bio-lipid would be biosurfactants which will be discussed later. Micro-organisms should not be primarily considered for producing oils and fats already available as they cannot compete with its cheapness. Micro-organisms though are very useful for handling waste materials such as starchy wastes, and the philosophy can be advanced that under these conditions it would be better to consider producing on oil, Single Cell Oil, instead of the much cheaper Single Cell Protein which is well-known but is only used for cheap animal feeding.

Microbial lipids are produced in abundance by a small group of oleaginous microorganisms. Of the 500 species of yeasts that are known only some 20 or so could be called 'oleaginous' by virtue of accumulating more than 20-25% of their biomass as lipid. How many filamentous fungi are also able to synthetise large amounts of oils and fats is unknown but probably are no more than a 100 or so. The structure of an oleaginous yeast differs from a common yeast cell. In many of the oleaginous fungi, potentially interesting fatty acids can be formed and some of these aspects are discussed later. The oil content may reach 70 % of the cell volume in some cases, most of it being accumulated in oil droplets. To achieve such high amount of oil, the medium has to be designed very carefully. The nitrogen source has to be exhausted at an early stage of the growth cycle and the sugar which remains in excess is then converted to triacylglycerol (triglyceride) oil. The predominant fatty acids are in most cases the same as found in plants with oleic acid and palmitic acid being predominant and usually being some C18:2 and C18:3 acids in addition (Table 1). Some organisms are able to produce a relatively high amount of a certain fatty acid but in many such cases the lipid content is usually rather low. Baker's yeast, for example, specifically accumulates palmitoleic acid (C16:1) but it does not produce much lipid and consequently does not allow an economic production.

Table 1. Lipid contents and fatty acids of some oleaginous organisms.

	Lipid content (% w/w)	Relative % of major fatty acids						
		16:0	16:1	18:0	18:1	18:2	18:3	22:0
Yeasts								
Candida curvata	58	32	-	15	44	8	1	-
Cryptococcus albidus	65	16	1	3	56	-	3	12
Lipomyces starkeyi	63	34	6	5	51	3	-	-
Rhodotorula glutinis	72	37	1	3	47	8	-	-
Trichosporon cutaneum	45	13	-	22	50	13	-	-
Moulds								
Aspergillus terreus	57	23	-	-	14	40	21	-
Fusarium oxysporum	34	17	-	8	20	46	5	-
Mortierella isabellina[1]	53(?)	27	1	6	44	12	8*	
Mucor javanicus[2]	20-22	24	1	6	40	11	18*	-
Pellicularia filamentosa	38	7	-	2	7	79	1	-

1 Production organisms used by J.S.E. Sturge (U.K.)

2 " " " " Idemitzer (Japan)

* GLA; other entries in this column are for α-linolenic acid

If a comparison is made between microbial oils and some commercial plant oils, a similar composition may be observed. In view of prices of for instance palm oil and other plant oils the conclusion has to be drawn that microbiologists have to identify and produce special, more valuable oils and fats in order to be able to compete with traditional products on the market. Three examples of such valuable oils are jojoba oil, cocoa butter, and the oil from Evening Primrose. Other examples include biosurfactants to be discussed towards the end of this presentation.

1. Jojoba oil

Jojoba oil is not a triacylglycerol but a wax and, with a certain degree of success, attempts have been made to produce a similar material using bacteria. However, it was soon realized that the bacterial product could not be sold as jojoba oil, simply because it was not jojoba oil. So no matter how good a substitute may be produced if the product you are competing against sells partly because of its name then there will be great difficulty selling something similar but without using that particular name.

2. Cocoa butter.

Cocoa butter owes its property - when it is incorporated into chocolate mainly because its high content of stearic acid. It has a combination of stearate, oleate and palmitate approximately in the combination 1:1:1 which gives the characteristic triacylglycerol. Now, the stearic acid content of most yeasts is fairly modest (5-10%) whereas in this instance a high content of stearic acid (25-35%) should be aimed at. So, the task is to increase the stearic acid content of the micro-organism. This task may be realized by:

- selection for a better producing micro-organism
- changing the cultivation conditions, e.g. substrate, pH, oxygen supply, temperature, etc.
- feeding stearic acid to the cells
- blocking the conversion of stearic acid to oleic acid either by selective inhibition or by mutation.

The first two approaches have so far not really been succesful in pushing up the amount of stearic acid in a micro-organism. The third strategy seems to be brilliant until it is realized that the stearic acid has to come from somewhere and that its price will be high. The last approach - with both variations - seems to offer a better solution.

Selective inhibition of the key enzyme, the Δ^9 desaturase has been achieved by Moreson in 1985, by using the naturally occurring cyclopropene fatty acid, sterculic acid. When sterculic acid was added in the form of sterculic oil having a 50 % sterculic acid content at a concentration of 0.5 mg/l the content of stearic acid in several yeasts was increased enormously (up to 35-40%) and consequently, the amount of oleic acid diminished. It is interesting to note that the synthesis of polyunsaturated fatty acids was hardly influenced and this was considered disadvantageous to the production of a satisfactory oil. Even so, the high level of stearic acid achieved was a tremendous break through and established that there was no intrinsic reason why a microorganism could not produce lipids with a very high content of saturated fatty acids.

The other strategy to produce a cocoabutter substitute is to delete by mutation the Δ^9 desaturase. This work was accomplished by a Dutch group headed by Nijkamp in Amsterdam with a Candida species. Many mutants were obtained and there were two that showed much higher levels of stearic acid (Table 2). But in these cases, the accumulation of stearic acid unfortunately led to a triacylglycerol with some of the stearic acid in the central 2 position which was not wanted. In spite of this, the potential seems to be very promising here, and it is probably just a matter of further investigation of these mutants in order to isolate successful organisms.

Table 2. Stearic acid levels in Candida.

Strain	Level of stearic acid (18-0) (% of total lipids)	Other acids 16:0	18:1	18:2
Candida wild type parent	14	28	44	10
Candida mutant [1]	52	22	10	7
Candida mutant partial revertant from mutant 1	41	26	16	8

The potentials of this yeast Candida curvata (=Apiotrichum curvatum), are now starting to be exploited in New Zealand for converting whey to single cell oil which has a much higher price than the ethanol which is produced at the moment using an alternative fermentation process. A process has been elaborated on based on a natural strain of this yeast with increased stearic acid content of up to 20%. According to present calculations, only a slight improvement in the yield is needed in order to achieve a sound return on capital investment.

3. Gamma-linoleic acid (GLA)

GLA (6,9,12-octadecatrienoic acid) is found in the seeds of Oenothera (Evening Primrose) and Ribes species. Various human disorders respond benificially upon daily intake of this GLA-rich oil. Finding alternatives for the economic production of GLA in the microbial kingdom would therefore be very useful. Some moulds have been known for over 40 years to produce GLA and thus would seem to be good candidates for investigation. Here there is a similarity in the composition of mould oils to those of plant sources. Preferred species of moulds are from the genus Mucor, Rhizopus, Mortierella and from a few other related organisms. In some strains, a very high GLA content was found but it was associated with a low level of lipid, while on the other hand, some strains showed very high lipid levels but with a low GLA content. The aim of our research at the University of Hull was to find a strain that could grow rapidly in submerged culture and would give at least 20% of total lipid and a minimum content of 20% GLA in that lipid. The systematic work shown schematically in Table 3 was successful and Mucor javanicus was identified as a production micro-organism fulfilling these criteria.

Table 3. Selection of GLA-producing species.

Method	Number of species after selection
screening 300 species (1 liter fermentor)	30
small scale fermentation (5 liter)	3
large scale fermentation (5000 liter)	1

The composition of the oil produced by Mucor javanicus has all the characteristics wanted as a substitute for the plant oils and moreover it is free of herbicide and pesticide residues which may not always be the case with the plant oils where the crops in the field are frequently sprayed with chemical control agents. The GLA content of the oil is 18% and may be pushed up to 22% and reaches thus the highest value found in plant oils.

This mould oil is now produced in 220 m^3 fermenters and the product, Oil of Javanicus, is marketed and sold in the U.K. and is available for distribution throughout the world. The mould product has some advantages over the plant products:

- the oil can be made on demand any time of the year;
- no storage and no deterioration has to be considered;
- the composition of the oil is unvarying and thus the quality and quantity can be absolutely guaranteed.

Other polyunsaturated fatty acids such as arachadonic acid (20:4), eicosopentaenoic acid (20:5) and docosahexaenoic acid (22:6) have also good prospects for commercialization. Mould technologies are being elaborated for these acids mainly in Japan. A huge potential market exists for these products but the take up will depend on whether cheaper alternatives (and non-technological) routes can be found.

4. Extracellular lipids

Extracellular lipids, e.g. glycolipids, are also under consideration for biotechnological production. Surfactants are the most important compounds belonging to this category. Many of these compounds have been isolated from various types of microorganisms but still a large number of them is expected to be found since the number of combination of sugars and fatty acids is almost endless. Their properties could be much different and very interesting for the commercial market.

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ENZYMATIC AND CHEMICAL MODIFICATION OF FATTY ACIDS

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Fatty acid molecules are rather resistant to chemical or enzymatic attack. They have a long hydrophobic tail with a carboxylic group on it. Three regions on the molecule can be distinguished to be susceptible to (bio)chemical modification. The sites and the types of reactions which are feasible on these sites are schematically depicted in figure 1. These reactions provide enormous possibilities to tailor fatty acids to specific molecules of interest for industrial application. Some examples of the routes where both enzymatic and chemical modifications are possible will be shown.

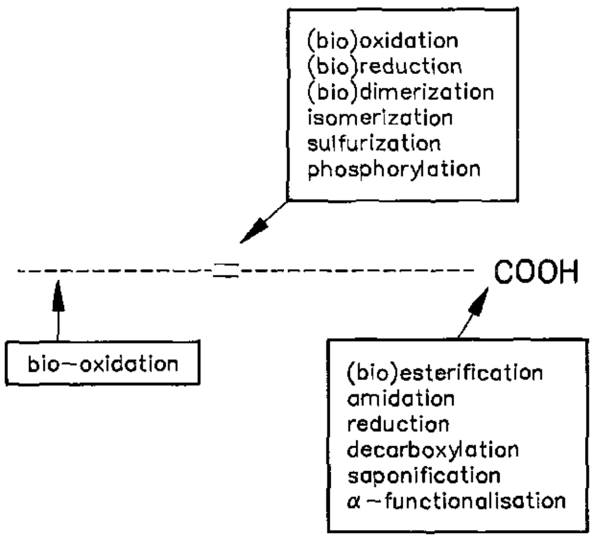

Figure 1. (Bio)chemical modifications of fatty acids.

1. (Bio)oxidation

1.1. Main biooxidation routes

The main biooxidation routes involve enzymatic reactions with lipoxygenases, alcohol dehydrogenases and oxidases, cytochrome P-450 systems and hydratases.

Lipoxygenase reactions

A lipoxygenase converts a fatty acid with at least two double bonds in a 1,4-pentadiene system to hydroperoxy-acids according to the scheme shown in figure 2.

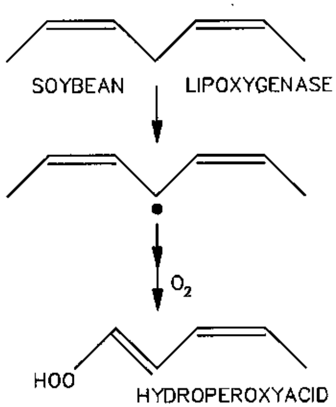

Figure 2. Lipoxygenase reactions.

Although not very stable, hydroperoxy-acids are important intermediates for producing very specific fatty acid dimers, hydroxy-acids, aldehydes or vinyl-ethers. The production of certain types of prostaglandins from the appropriate polyunsaturated fatty acids is an other interesting example.

Cytochrome P-450 sytems

These systems can oxidize the hydrophobic end of a fatty acid molecule or the double bond(s). In Bacillus megaterium either an epoxide or unsaturated hydroxy-acids can be formed in this way (Fig. 3). Only limited work has been done on this route, but the results are very interesting. In yeast, oxidation is performed by subsequent reaction steps of the cytochrome P-450 system to yield dicarboxylic acids which normally are further oxidized via beta-oxidation. By blocking this route, the accumulation of dicarboxylic acid can be achieved up to 100 g/l. By chemical means ω-oxidation is not yet feasible. The reaction is given schematically in figure 4. The dicarboxylic acids find many applications in lubricants, plasticisers and as esters. Epoxy fatty acids as obtained by the Bacillus cytochrome P-450 system have wide applications mainly as plasticisers.

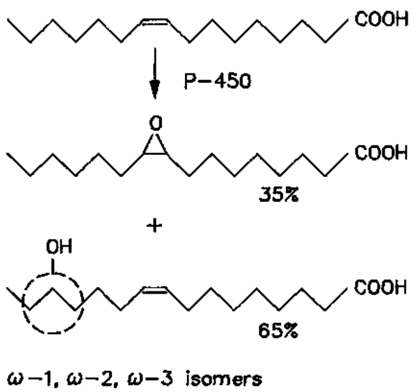

Figure 3. Oxidation of a double bond or of a hydrophobic end.

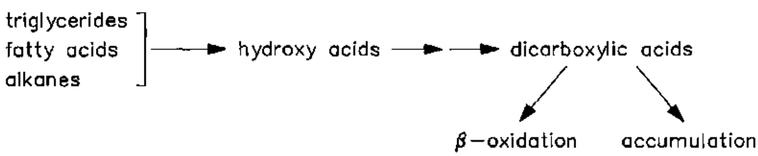

Figure 4. Accumulation of dicarboxylic acids.

Alcohol dehydrogenase and oxidases reactions

Secondary alcohol dehydrogenases and oxidases of several microorganisms can oxidize hydroxy-fatty acids to keto-acids. The reaction may be performed by two different types of enzymes (Fig. 5) where the oxidase reaction using oxygen as cosubstrate is more attractive than the dehydrogenase reaction with NAD+ or methyl-viologen as cosubstrate.

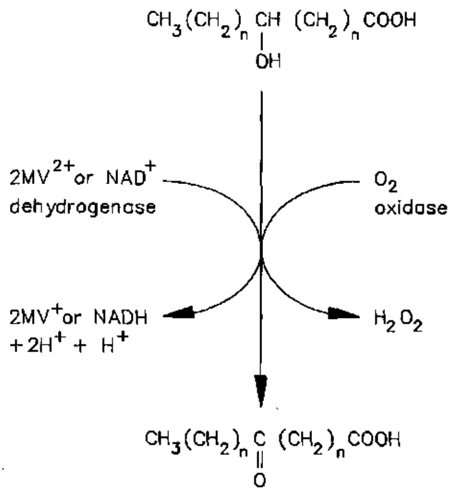

Figure 5. Oxidation of secondary alcohols.

Hydratase reactions

Hydratases are capable of introducing a stereospecifically hydroxo-function to the double bond of a fatty acid. There are several types of it in various microorganisms e.g. in Claviceps, Pseudomonas and Corynebacterium species. The best studied enzyme is oleate hydratase which forms the R-10-hydroxy product from oleic acid. Applications for such hydroxy-fatty acids are quite broad - because of the additional functional group - as greases, surface coatings, food thickeners and cross linking agents.

1.2. Main chemical oxidation routes

The main targets for chemical oxidation reactions are the double bond, the allylic position which is slightly activated by the double bond, the α-carbon atom and in some cases also the β-carbon. The most frequent reactions are given in figure 6, while in figure 7 a scheme is given for reactions performed at the double bond.

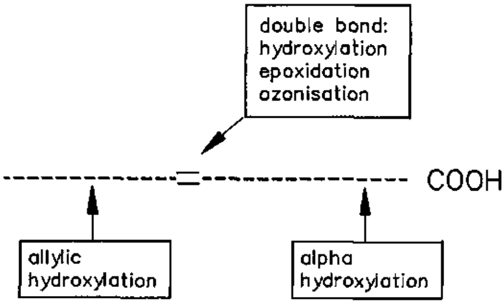

Figure 6. Chemical oxidation reactions.

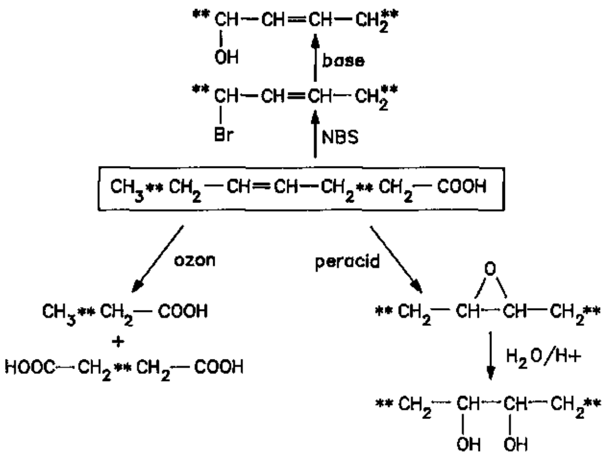

Figure 7. Chemical reactions at the double bond.

2. (Bio)reduction

2.1. Main bioreduction routes

Bioreductions are mainly performed by saturases in an NADH or NADPH dependent site specific fashion. Starting from polyunsaturated fatty acids, rumen bacteria are capable of performing reactions given in figure 8. Several interacting bacteria are known to be involved in the pathways shown. The exact mechanism remains however obscure.

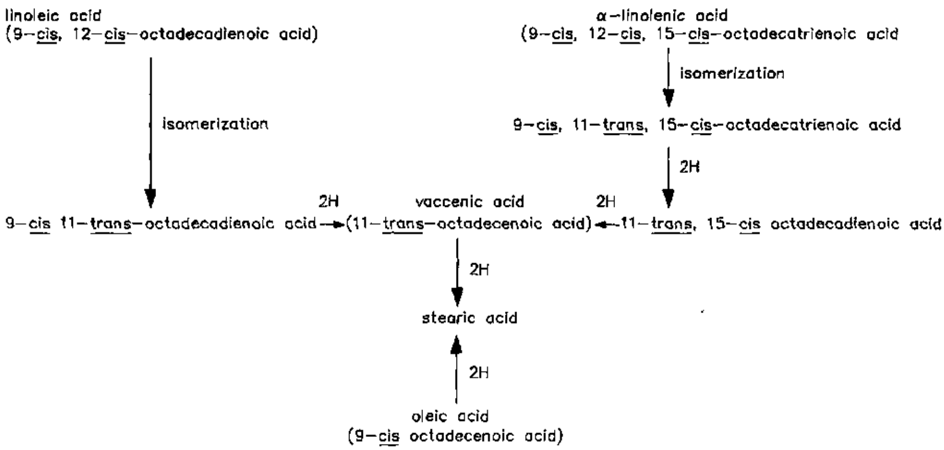

Figure 8. Bioreduction reactions by rumen bacteria.

2.2. Main chemical reduction routes

The chemical reduction of unsaturated fatty acids is relatively easy. Either the double bond or the carboxylic group may be reduced in a metal-catalyzed reaction. The biohydrogenation route offers some advantages over the chemical one but it will probably take a long time before such a process can be commercialized.

3. (Bio)dimerization

3.1. Enzymatic process

Dimer acids have a relatively low melting point and have a smooth feel. They are used in cosmetic creams. Employing lipoxygenase activity it is possible to obtain dimer acids from unsaturated acids. The route is convenient for producing specific linear dimers. The yields are, however, rather low.

3.2. Chemical dimerization

For chemical dimerization a clay catalysed reaction is applied by most companies, also by Unichema. This reaction leads to a whole range of different types of products. The structure of the branched fatty acids formed show a great variety with the main type of branching occurring around the site of the original double bond. In contrast to the enzymatic way, here a lot of different dimers and also some trimers are formed. They have aromatic, cyclic or linear structures, their ratio depending on the reaction conditions. The biological and chemical dimerization reactions are summarized in figure 9.

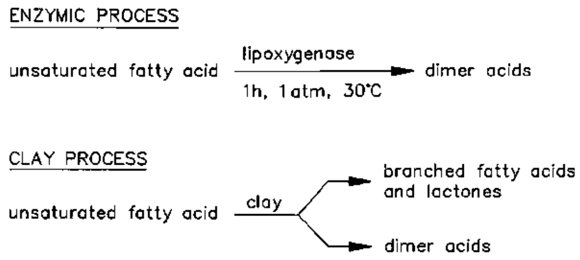

Figure 9. Biological and chemical dimerization reactions.

There are of course far more opportunities for enzymatic and chemical modifications, i.e. reactions performed on isolated fatty acids. It would be nice if the plant breeder could manage to let the plant itself do the chemistry instead of the chemist.