Grasas y Aceites
Vol. 49. Fasc. 3-4 (1998), 303-309

The formation of new compounds

By **A. Gasparoli**

Stazione Sperimentale per le Industrie degli Oli e dei Grassi
20133 – Milano, Italy

SUMMARY

The formation of new compounds

The study of model systems allows us to reconstruct the complex mosaic of reactions which make up the frying process. In particular such systems, if made up of single oils or their components, verify the transformation of the triglyceride matrix by thermo-oxidizing treatment. Should this then have added specific substrates (protein, starch) they reconstruct the oil-substrate interaction and the influence of the same substrate in the transformation. The contribution of the information is equally distributed between the volatile products and the complex products; in fact both contribute to indicate the reactions in play, supporting the previous considerations for investigation at an analytical level. The present work discusses the newly formed products (volatile or nonvolatile), verifying in particular the formation of reaction products with the substrates.

KEY-WORDS: Heated fat – Polar compounds – Polymer compounds – Substrates.

Thermo-oxidative treatment modifies the triglyceridic structure of an oil, determining the formation of new compounds, generally classified as volatile or nonvolatile, depending on their persistance in the mass of the reaction.

In the process the operative variables (frying time, temperature, nature of the oil, type of food and technology used) are fundamental: their influence determine the predominance of one chemical reaction over another.

The first phase of the process consists in heating the oil at working temperature.

Based on this parameter a series of newly compounds are formed (Figure1).

OIL --------- frying ---------
POLYMER COMPOUNDS
OXYDATED TRIGLYCERIDES
VOLATILE COMPOUNDS

OIL + SUBSTRATE -- frying ---
POLYMER COMPOUNDS
OXYDATED TRIGLYCERIDES
VOLATILE COMPOUNDS
COMPLEXE COMPOUNDS

Figure 1
Compounds originated by frying

When the food is introduced, alongside these products, whose concentration increases depending on the variables already indicated (time, temperature, nature of oil, type of frying), we assist in the phenomenon of oil - food interaction which leads to the formation of even more complex products, (Figure 1), linked above all to the nature of the food used.

Based on the above mentioned, which determine how many and which modifications can take place, it resulted useful for us to simplify the process, turning to the study of the behaviour of a systems model, on which to verify the chemical effects of the process, at least in a qualitative way.

Thermo-oxidizing an oil, a simplified system model, referable to the initial phase of the frying process, revealed the increase of a peak obtained in particular working conditions. The intensity of this peak results proportional to the time of treatment, as can be seen on the HPLC (Figure 2) chromatogram (Gasparoli, 1986).

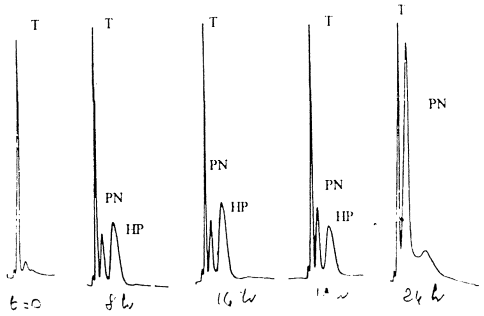


Figure 2
HPLC analysis (230 nm) of oil during Frying

T = unaltered triglycerides
PN = neoformation products
HP = hydroperoxides

The t_R HPLC, the λ used (230nm), induced us to assume that, for this peak, a triglyceride nature must be slightly modified by the inclusion of a function in the fatty acid molecule or by a slightly heavier molecular weight.

Two paths were followed to arrive at the identification of the chemical nature of such a peak and both paths led to a significative result.

The use of chromatography columns on cellulose (Figure 3), consented the isolation of a fraction, with PM superior to that of triglycerides, which present the following characteristics: maximum absorption in UV at 232 nm, as noted in the HPLC analysis; infrared spectra shows the absence of functional polar groups; gaschromatographic chromatogram (before transmetilation) which, based on the working conditions adopted (short columns with apolar phases), showed the presence of dimers.

Consequently, we hypothesize that there is a dimerization present in the molecule which justifies the polarity found and that both steric and positional isomerization occured in the molecule.

In effect the gaschromatographic analysis on capillary columns (Osella S.) evidenced a notable complexity in the components (Figure 4).

The analysis for mass spectrometry revealed the presence of two principle components, for the first we hypothesize the origin for the reaction between linoleic and linolenic acid.

The typology of the resulting chromatogram indicates the presence of possible isomerisms of the dimer.

The interpretation of the second peak is more complex and its structure is under study.

The monomer fraction supplies further and significative information from which the polar components are investigated.

In particular, a fraction became isolated and this also presents chromatographic and spectral (UV) characteristics of the dimer fraction, but a different IR spectrum and different gaschromatographic elution (Figure 5).

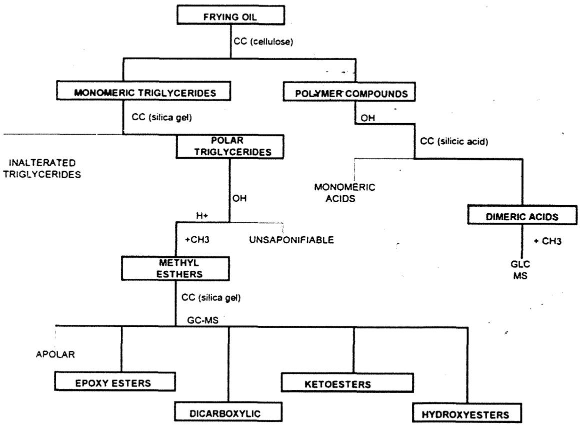


Figure 3
Schema for components separation of fryting oil

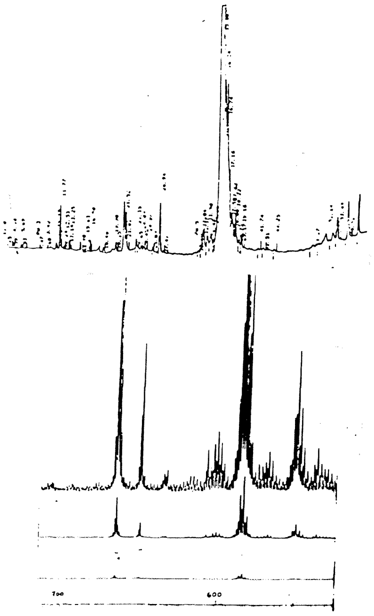


Figure 4
HRGC separation of PN components derived from silicic acid separation: dimeric acids fraction and mass-spectometry

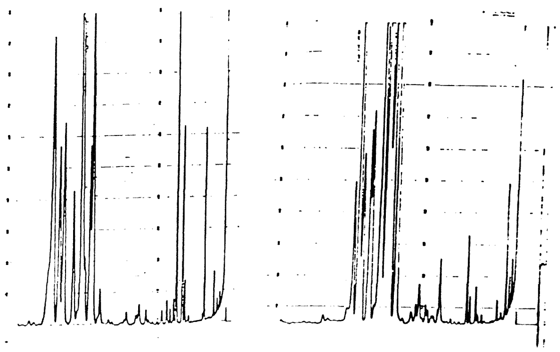


Figure 5a
HRGC chromatograms of two fractions of epoxy compounds (separated by TLC)

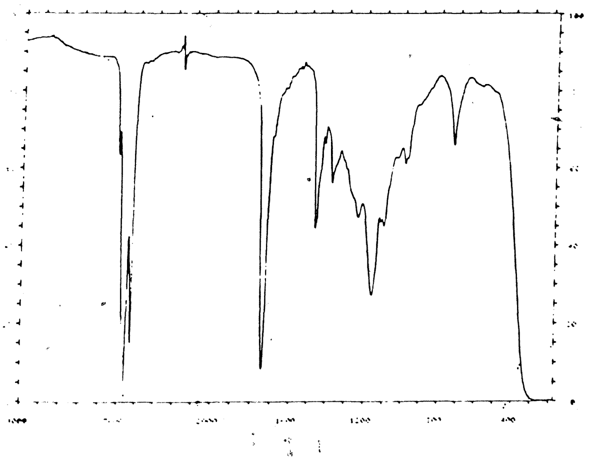
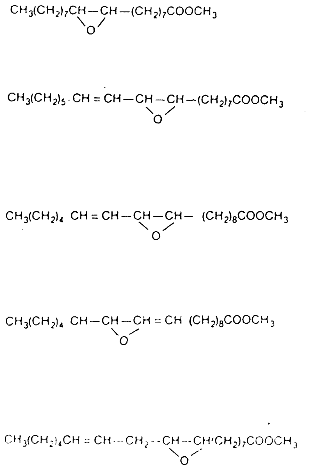


Figure 5b
IR spectra of fraction epoxy compounds

Based on previous experience, the gaschromatographic conditions used led us to assume the presence of epoxides (Gasparoli, 1986).

This was confirmed by successive analysis for GC-MS, which detect the compounds in Tab. I.

TABLE I
Epoxy compounds identified by GC-MS



$CH_3(CH_2)_7\underbrace{CH-CH}_{O}-(CH_2)_7COOCH_3$

$CH_3(CH_2)_5 \cdot CH=CH-\underbrace{CH-CH}_{O}-(CH_2)_7COOCH_3$

$CH_3(CH_2)_4\ CH=CH-\underbrace{CH-CH}_{O}-\ (CH_2)_8COOCH_3$

$CH_3(CH_2)_4\ \underbrace{CH-CH}_{O}-CH=CH\ (CH_2)_8COOCH_3$

$CH_3(CH_2)_4CH=CH-CH_2-\underbrace{CH-CH}_{O}(CH_2)_7COOCH_3$

In particular the presence of both treo and erythro forms can be assumed as through TLC two bands of different chromatographic elution with identical fragmentations are obtained.

A general indication on the nature of the more polar compounds is supplied by the analysis by IR spectrophotometry, from which the functional groups present, in particular ketonic and bicarboxylic groups, can be identified.

The gaschromatographic analysis evidences well the complexity of the single fractions obtained by chromatographic separation; for GC-MS the more significative components of such fractions are identified (Grillo, Stronati) and these prove to formed of saturated and mono-unsaturated bicarboxylic acids, saturated and mono-unsaturated ketoesters, of saturated and unsaturated hidroxyesters (Tab. II).

To complete the picture of the rearrangement and to consent a partial interpretation of the mechanisms which are present in the phenomenon of frying, the volatile component must be examined.

The importance of these compounds is not so much to be found in their contribution to the modification of the original organoleptic characteristics as in the possible help which may be supplied for the interpretation of the complex reactions which occur.

In the thermic treatment of an oil and of its components the compounds shown in Tab.III (Fedeli, 1974, 1976) were identified. These were precisely saturated and unsaturated aldehydes, hydrocarbons, ketones, fatty acids, lactones, alcohols.

TABLE II
Polar compounds identified by GC-MS

DICARBOXYLIC ESTERS

$CH_3COO(CH_2)_6COOCH_3$ and monounsatured
$CH_3COO(CH_2)_7COOCH_3$ and monounsatured
$CH_3COO(CH_2)_8COOCH_3$ and monounsatured
$CH_3COO(CH_2)_9COOCH_3$ and monounsatured
$CH_3COO(CH_2)_{10}COOCH_3$ and monounsatured
$CH_3COO(CH_2)_{11}COOCH_3$ and monounsatured

KETOESTERS

$CH_3(CH_2)_8$-CO-$(CH_2)_7COOCH_3$
$CH_3(CH_2)_4$-CO-$C_{11}H_{18}COOCH_3$
$CH_3C_8H_{12}$-CO-$(CH_2)_7COOCH_3$
$CH_3(CH_2)_2$-CO-$(CH_2)_4COOCH_3$
$CH_3C_6H_{10}$-CO-CH_2COOCH_3
CH_3-CO-$(CH_2)_8COOCH_3$
$CH_3C_7H_{12}$-CO-CH_2COOCH_3
$CH_3(CH_2)_2$-CO-$(CH_2)_3COOCH_3$
CH_3-CO-$(CH_2)_7COOCH_3$

HIDROXYESTERS

CH_3COO-HCOH-$(CH_2)_{13}CH_3$
$CH_3\ C_{15}H_{28}$-HCOH- COO CH_3
$CH_3\ C_{11}H_{18}$-HCOH- $CH_2COO\ CH_3$

TABLE III
Volatile compounds found in frying seed oil

Hydrocarbons	Ketones	Aldehydes
Toluene	Methyl pentyl ketone	C4
Ethylbenzene	C8 uns	C5
n-buthylbenzene	C11 methyl ketone	C6
iso buthylbenzene	C10 methil ketone	C7
1 methyl 2 propylbenzene	C6 uns	
Alcohols	**Acetals**	**Eterocyclic**
C4	Dietoxy uns ald. C6	2 methylfurane
C5		Dihydropyrane
C7		2 Ethyl furane
C8		2 Buthyl furane
C9		2 Propyl furane
C10		2 Pentyk furane

In the real thermo-oxidizing process the components of a complex system interact.

These are made up of not only of the oil, but also the food substance, whose chemical composition may influence the complex paths of rearrangement of the triglyceride at high temperatures, complicating the resulting analytical picture.

In order to simplify the study, model systems made up of oil added to the base component of the food were used. These were starch (to simulate the frying of potatoes) and amino acids (to simulate the frying of meat products).

In the case of the treatment with starch, the transformation induced in the non volatile component led, in part, to the formation of products of oxidation and, in part, to the formation of polymers, as shown in IR spectrums carried out on the fractions separated by columns.

Particularly interesting in this phase resulted the evaluation of the residue from which, for extraction with methyl alcohol and successive fractionation by TLC, a series of compounds which seem to represent products of reaction between starch and lipid material (Figure 6).

The presence of absorption characteristic of triglycerides, accompanied by absorption attributable to the starch free oxidriles and others, attributable to the etherification of the hydroxyl groups of starch with the fatty acids, which occured.

Less specific the situation of compounds identified in the volatile fractions reported in Table IV.

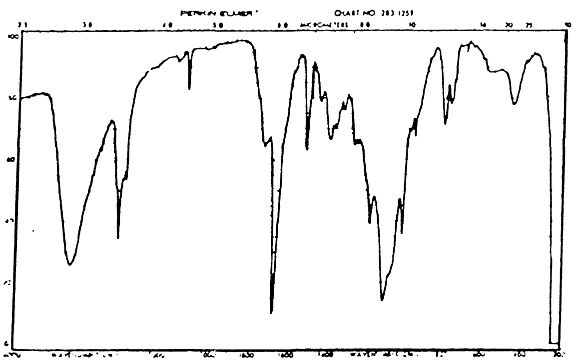
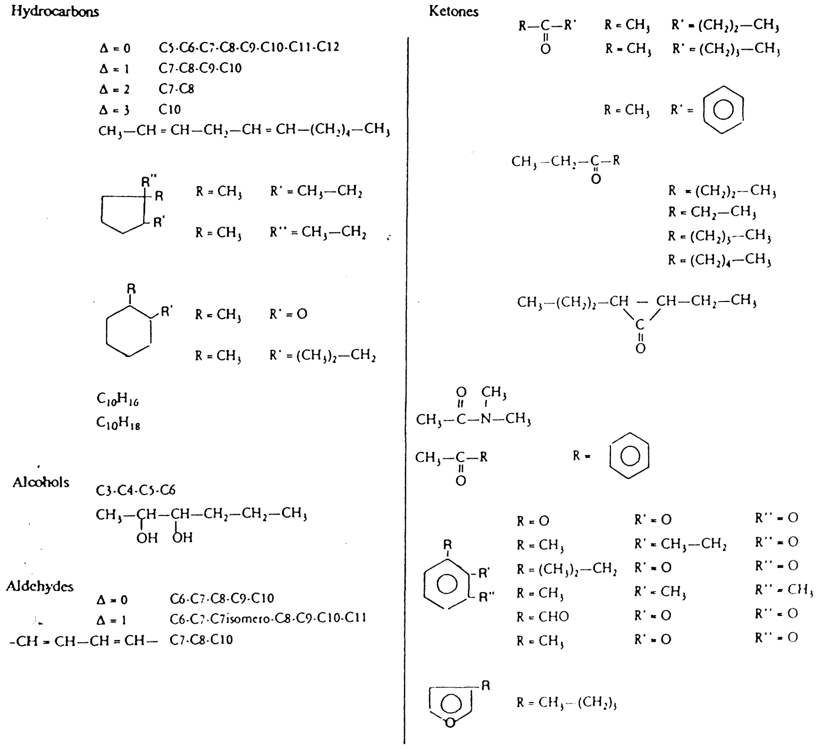


Figure 6
IR spectra of methanolic extract obtained after frying of oil added of starch

TABLE IV
Volatile compounds identified by GC-MS in frying soyabean oil with starch



Hydrocarbons

$\Delta = 0$ C5-C6-C7-C8-C9-C10-C11-C12
$\Delta = 1$ C7-C8-C9-C10
$\Delta = 2$ C7-C8
$\Delta = 3$ C10
$CH_3{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}(CH_2)_4{-}CH_3$

$R = CH_3$ $R' = CH_3{-}CH_2$
$R = CH_3$ $R'' = CH_3{-}CH_2$

$R = CH_3$ $R' = O$
$R = CH_3$ $R' = (CH_3)_2{-}CH_2$

$C_{10}H_{16}$
$C_{10}H_{18}$

Alcohols

C3-C4-C5-C6
$CH_3{-}CH(OH){-}CH(OH){-}CH_2{-}CH_2{-}CH_3$

Aldehydes

$\Delta = 0$ C6-C7-C8-C9-C10
$\Delta = 1$ C6-C7-C7isomero-C8-C9-C10-C11
$-CH{=}CH{-}CH{=}CH-$ C7-C8-C10

Ketones

$R{-}C({=}O){-}R'$
$R = CH_3$ $R' = (CH_2)_2{-}CH_3$
$R = CH_3$ $R' = (CH_2)_3{-}CH_3$
$R = CH_3$ R' =

$CH_3{-}CH_2{-}C({=}O){-}R$
$R = (CH_2)_2{-}CH_3$
$R = CH_2{-}CH_3$
$R = (CH_2)_3{-}CH_3$
$R = (CH_2)_4{-}CH_3$

$CH_3{-}(CH_2)_2{-}CH{-}CH{-}CH_2{-}CH_3$

$CH_3{-}C({=}O){-}N(CH_3){-}CH_3$

$CH_3{-}C({=}O){-}R$ R =

R	R'	R''
$R = O$	$R' = O$	$R'' = O$
$R = CH_3$	$R' = CH_3{-}CH_2$	$R'' = O$
$R = (CH_3)_2{-}CH_2$	$R' = O$	$R'' = O$
$R = CH_3$	$R' = CH_3$	$R'' = CH_3$
$R = CHO$	$R' = O$	$R'' = O$
$R = CH_3$	$R' = O$	$R'' = O$

$R = CH_3{-}(CH_2)_3$

These products could derive from the combination of free radicals, present on the glycerides, with starch.

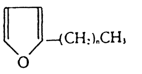
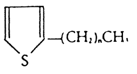
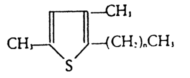
Treatment with aminoacids leads to identify of specific volatile compounds (Tab. IV) alongside products presumably deriving from the degradation of the matrix used (oil or its specific components).

From the general picture that is obtained, that is :

— fewer polymer substances
— more polar substances
— fewer volatile compounds
— specificity of volatile compounds
— typology of polar products

it can be considered that the aminoacids intervene to modify the mechanism of the implicated reactions; in particular a reduction in newly formed products of a polymeric nature was noted.

TABLE V
Volatile compounds identified by GC-MS of oil frying with amminoacids

		+ Met	+Cys
Hydrocarbons	Δ = 0	7,8,9,12,13,14,15,16	11,12,13,14,20,21
	Δ = 1	6,7,8,9,12,14,15,16,17	14
	Δ = 2	13	
C6H11R		R= (CH2)8 CH3	R=CH3, CH2CH3
C6H9(CH2)nCH3		n= 1,2,3,4,5,6,7,8	n=2,3,4,5,6,7,8
Aldehydes	CH3(CH2)nCHO	n= 3,4,5,6,7,8,9	 
Ketones	CH3COCH(CH3)2		n = 6,7 n = 1 n = 3,4
Fatty acids	Δ=0	4,9,14	 n = 5,8 n = 2,5,7
	Δ =1	10,11,12,15	
	Δ=2	12,16,18	
		DIMETHYLSULFIDE	

The most significant volatile product, (Fedeli, 1976, 1979) is the dimethylsulfide, isolated in the reaction tests with methionine, as it identifies a marked decomposition of the aminoacid itself, also verifiable at relatively low temperatures (135°C).

This is also confirmed by the presence of CS and NH_2 groups in the nonvolatile fraction.

In this both the fractioning of the polar substances and the fractioning of the saponified products allow for interesting deductions.

The corresponding IR spectrums indicate that an interference on the aminoacids has occured in the reaction mechanisms, leading to the formation of nitrogen derivatives.

One part of these compounds could derive from the direct copulation of the aminoacids with the fatty acids in one position of the carbon chain, giving products deriving from the suppression of a radical formed during the auto-oxidating reaction of the fatty acids.

Another part could derive from the reactions that involve the carboxyl of the fatty acids and the aminic group of the aminoacids, also presumably forming cyclic compounds.

In the case of treatment with alanine the resulting product appears more linear, since it does not imply the presence of further bonds due to the SCH_3 group present in metionine: therefore each aminoacid can give rise to diversified mechanisms, while still referring to a common fundamental reaction mechanism.

A further verification of the influence of aminoacid and its direct intervention were obtained following the behaviour of two oils of the same composition (sunflower at high oleic) but with a different refining treatment, thermally treating both of them at 180°C and adding 1% of metionine, cysteine, alanine.

The chromatographic results obtained (Figure 7 compare oil tq, oil before frying, oil frying with metionine, alanine, cysteine) can see the different behaviour to frying in presence of aminoacids, particulary in presence of metionine.

This grafic (Figure 8) compare the different kinetics between oil with and without metionine in the formation of new components.

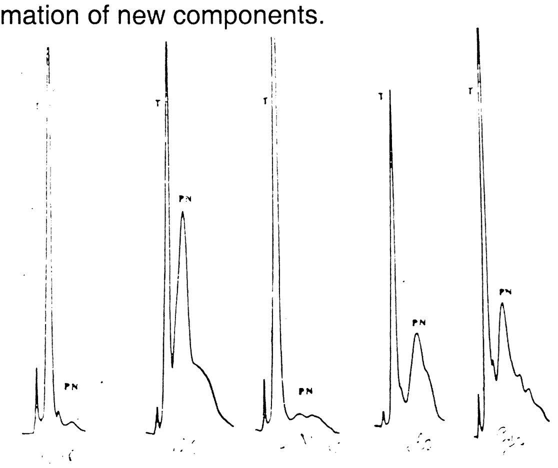


Figure 7
HPLC chromatograms of oil frying

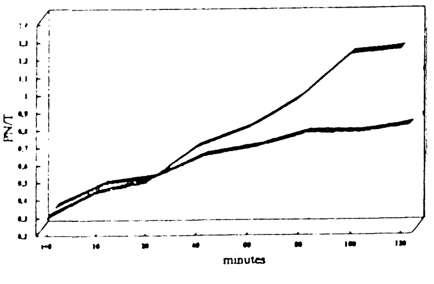


Figure 8
Kinetic of oil frying tq and added with methiodine

Different wose been the behaviour of oil with high content of hydroperoxides (Figures 9-10): the intervent of metionine is less incisive in the inibition in the formation of new component, but their influence leads to the formation of secondary products of oxidation, in similar way to occur in oil added with colours iron based (Gasparoli, 1994).

This would appear to confirm, at least in a preliminary way, the hypothesis of an ox - redox mechanism.

Evaluating the quantitative data through which the evolution of HP and SP were followed can be see reduction in the HP corresponding to an increase in the SP (this is clearly evident in Figure 11).

It is, however, evident, also because of the difference in colour to the other samples, that some other phenomenon has occured in the presence of metionine.

An even more simplified model system, made up of erucic acid with the addition of 1% of the aminoacids metionine, alanine and cystein, are submitting to thermic treatment (180°C for 6 hours).

The fractioning of reaction products on columns of silicic acid leads to the isolation of products whose IR spectrum, can identify the presence of compounds of a amidic nature (Figure 12). Some fractions are submitted to gaschromatography-masspectrometry: amongst the products present a cyclic amide and erucic acid amide are identified (Figures 13-14).

In conclusion, the positive aspect of the use of proteic material in the frying process is connected to the inhibition of dimeric products, formed by the reaction between glycerides or free fatty acids.

To this reality corresponds the formation of equally complex products, determined by the lipid-protein interaction.

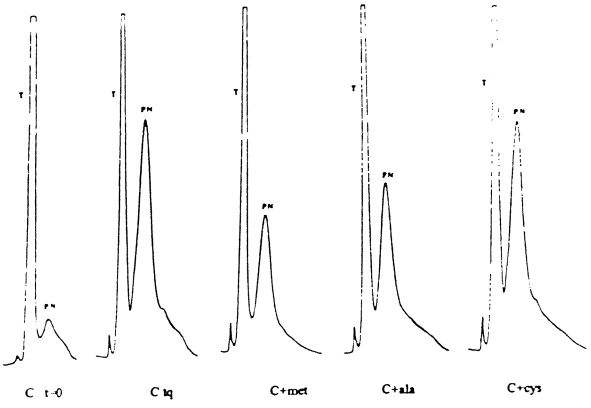


Figure 9
HPLC chromatograms obtained from oil after frying with aminoacids

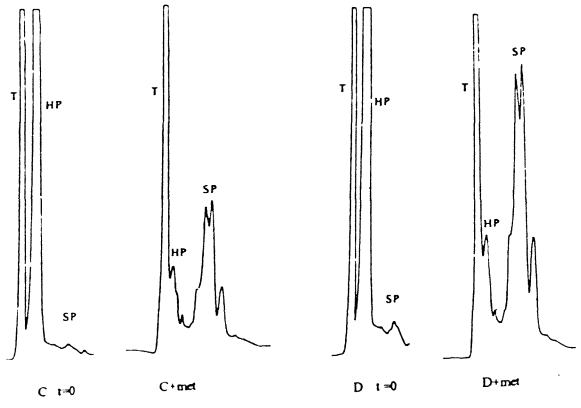


Figure 10
HPLC chromatograms related to transformation hydroperoxides of oil C and D after frying with methiodine

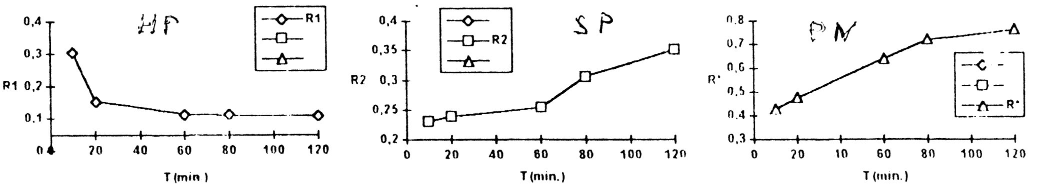


Figure 11
Graphic explained the behaviour of PN, HP, SP in the frying process with methionine

In particular, the simplification of the process using a systems model has allowed us:

1. to identify analytically the inhibited products: the HPLC analysis demonstrated a reduction in the formation of PN based on the presence of aminoacids and of the type of aminoacid used;

2. to confirm the importance of the quality (intended both as level of rectification process and

as state of oxidation) of the lipid present in the process. Indeed complex reactions occur in which, the more inferior the quality of the oil, the aminoacid can intervene only partially;

3. the investigation of the nonvolatile fraction has allowed us to complete the picture of the mechanisms of reaction, previously hypothesized through the study of volatile compounds formed by thermic treatment with aminoacids.

The finding of dimethylsulfide in the volatile fraction of the model systems allowed us to hypothesize that the decomposition of the metionine occurs in a complex mechanism in which the radical reaction should be predominant; as indicated by other authors (Sims,1975), a decarboxylation occurs, and, in presence of carboxyl acids, for the loss of a molecule of water, a cyclic amide is obtained.

Such behaviour was also verified on real fried food to indicate an objective correspondence in the analytical-structural evaluation of the oil - food substance interaction.

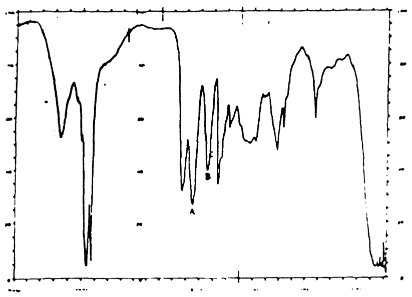


Figure 12
IR spectrum of compounds isolated after reaction erucic acid-methionine

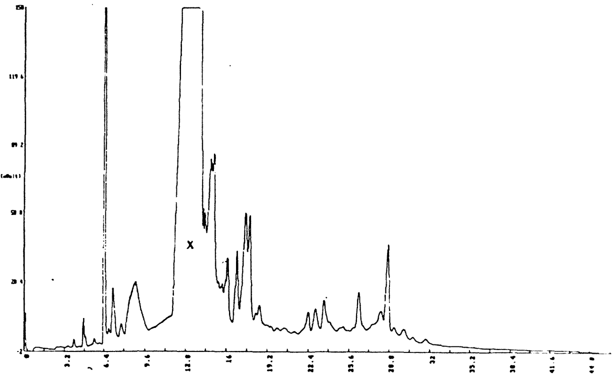


Figure 13
HRGC chromatogram obtained from compounds isolated (Figure 12)

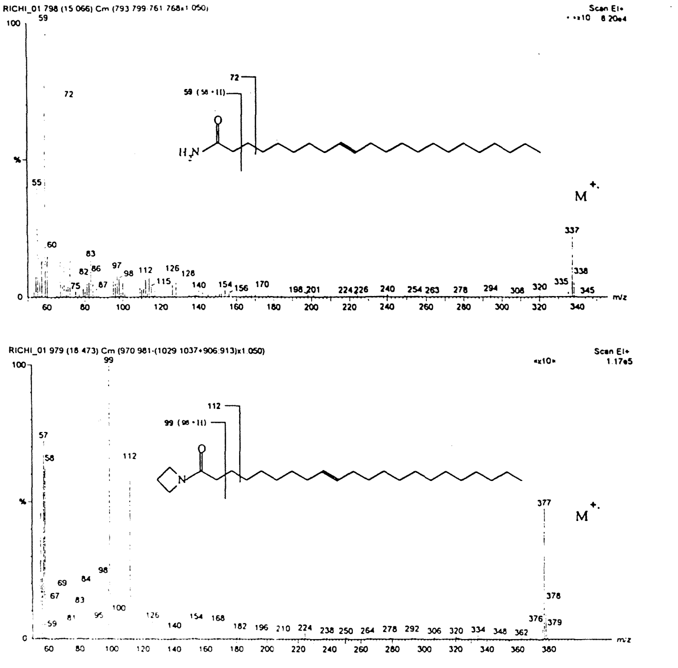


Figure 14
Fragmentation obtained by mass spectrometry of fraction isolated by column chromatography

REFERENCES

Billek, G., Guhr, G., Wabel, G. (1978).—«Quality assestment of used Frying Fats: a comparision of four methods».—J. Am Oil Chemist's Soc. **55**, 728-733.

Fedeli, E., Gasparoli, A., Favini, G., Daghetta, A. (1974).—«Trasformazioni degli oli vegetali per riscaldamento in presenza di aria. Nota I: olio di oliva e di arachide».—Riv. It. Sost. Grasse, vol. LI, 103-110.

Fedeli, E., Gasparoli, A., Favini, G., Daghetta, A. (1976).—Lipids, vol. 2º, ed. Paoletti R., Jacini, G., Porcellati, R.—Raven Press; New York, 491-495.

Fedeli, E., Gasparoli, A., Favini, G., Daghetta, A. (1976).—«Trasformazione degli oli vegetali per riscaldamento in presenza di aria. Nota III: Interazione tra trigliceridi dell'olio d'oliva e aminoacidi».—Riv. It. Sost. Grasse, vol. LIII, 7-10.

Fedeli, E., Gasparoli, A., Daghetta, A.—«Trasformazione degli oli vegetali per riscaldamento in presenza di aria-Nota IV: Indagine sufla trasformazione degli oleati di metile ed etile e dei loro idroperossidi per effetto termico».—Riv. It. Sost. Grasse, vol. LIII, 282-288.

Gasparoli, A., Mariani, C., Fedeli, E. (1986).— «Identificazione e dosaggio di alcuni composti di neoformazione originati nel trattamento termoossidativo degli oli alimentari».—Riv. It. Sost. Grasse, vol. LIII, 383-392.

Gasparoli, A., Fedeli, E. (1994).—18º IFSCC Congress «the cosmetic image: a mosaic of biosciences».—Venezia, 794.

Grillo, F., Anno Accademico 1983-1984.—«Identificazione di composti polari di neoformazione derivanti dal trattamento termico di oli alimentari».—Tesi di laurea in Scienze delle preparazioni alimentari-Milano.

Osella, S. Anno Accademico 1990-1991.—«Cottura degli alimenti ad alta temperatura. Aspetti tecnologici e di base».—Tesi di laurea in Scienze delle preparazioni alimentari -Milano.

Sims, R.J., Fioriti, J. A. (1975).—J. Am. Oil Chemist's Soc. **55**, 728-733.

Stronati, R., Anno Accademico 1984-1985.— «Identificazione di composti di neoformazione originatisi durante il trattamento termico degli oli alimentari».—Tesi di laurea in Scienze delle preparazioni alimentari-Milano.