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REVISIÓN

Aldehydic acids in frying oils: formation, toxicological significance and analysis

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RESUMEN

Acidos aldehídicos en aceites de fritura: formación, significado toxicológico y análisis.

Acidos aldehídicos son producidos en lípidos oxidados como resultado de la descomposición de hidroperóxidos por reacciones de β-escición. Es conocido que los aldehídos interaccionan con las proteínas y el ADN y debilitan las funciones enzimáticas. Los ésteres aldehídicos de lípidos oxidados fueron reabsorbidos en una cantidad significativa en ratas. Este artículo revisa los mecanismos de formación de ácidos aldehídicos esterificados en aceites de fritura y sus efectos fisiológicos/toxicológicos. El artículo también ofrece una visión de conjunto de las técnicas analíticas básicas que necesitan ser mejoradas para establecer métodos cuantitativos fiables.

PALABRAS-CLAVE: Aceite de fritura – Ácido aldehídico – Análisis – Formación – Revisión (artículo) – Síntesis – Toxicidad.

SUMMARY

Aldehydic acids in frying oils: formation, toxicological significance and analysis.

Aldehydic acids are generated in oxidized lipids as a result of decomposition of hydroperoxides by β-scission reactions. Aldehydes are known to interact with proteins and DNA and to impair enzymic functions. Aldehydic esters from oxidized lipids were resorbed to a significant extent in rats. This paper reviews the mechanism of formation of esterified aldehydic acids in frying oils and their physiological/toxicological effects. The paper also gives an overview of relevant basic analytical techniques that needs to be improved to establish reliable quantitative method (s).

KEY-WORDS: Aldehydic acid – Analysis – Formation – Frying oil – Review (paper) – Synthesis – Toxicity.

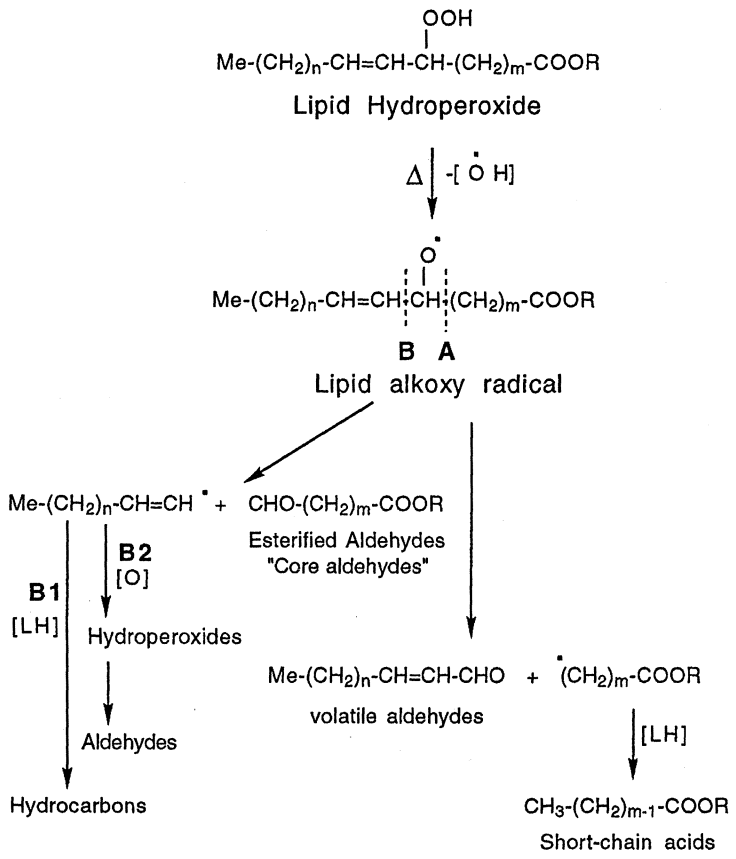
1. INTRODUCTION

During deep-fat frying, a wide number of changes take place in oils and fats as a result of complicated thermolytic and oxidative reactions (Chang & Peterson, 1978; White, 1991). Among a number of secondary oxidation products, two types of aldehydes (viz., volatile aldehydes and esterified aldehydic acids) are produced from acyl lipid hydroperoxides by β-scission reactions. The volatile aldehydes have recieved much attention due to their implications in rancid odours and flavours (see e.g. Frankel, 1982, 1985a). The esterified aldehydes, on the other hand, are of particular interest since they are retained in the frying oil which is absorbed by the fried food and hence ingested by the consumer (Smith *et al.*, 1986; Chanin *et al.,* 1988).

This paper reviews the formation of esterified aldehydic acids in frying oils and their physiological/toxicological effects and gives an overview of relevant basic analytical techniques that needs to be improved to establish reliable quantitative method (s).

2. THE FORMATION OF ALDEHYDIC ESTERS IN FRYING OILS

The formation of hydroperoxides as primary oxidation products from unsaturated fatty esters is well documented and reviewed (Frankel, 1985a,b, 1991). On homolytic thermal decomposition, the hydroperoxides decompse to alkoxy radicals which then undergo β-scission on either side of the alkoxy carbon through route A or B (Scheme 1) to produce two types of aldehydes (volatile aldehydes or esterified aldehydic acids) and an alkyl or alkenyl radical. Whether the scission would proceed through route A or B, depends on the nature of the primary hydroperoxide and on temperature. Generally, scissions creating alkenyl radicals are energetically less favourourable because the heat of formation and related bond dissociation energy required for the formation of a vinyl radical is larger than that needed for the formation of an alkyl radical (Frankel & Gardner, 1989). It should also be noted here that the alkenyl radicals, when formed, are very reactive and unstable. They undergo spontaneous reactions with available radicals and/or with oxygen. The later reaction may lead to further formation of aldehydes (Frankel, 1985b).



Scheme 1
The formation of aldehydic acid esters from lipid hydroperoxides

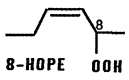
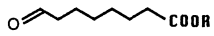
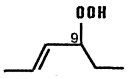
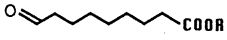
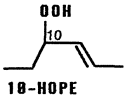
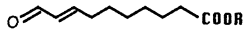
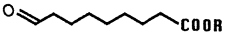
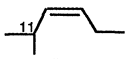
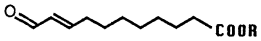
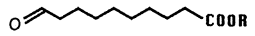
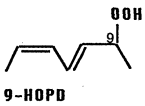
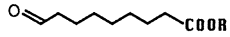
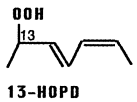
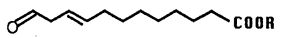
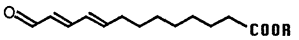
Since frying oils contain insignificant amounts of linolenic acid (18:3), the discussion will be focused on the oxidation products from oleic (18:1) and linoleic (18:2) acids. The oxidation of oleate yields four types of hydroperoxides, namely: 8-hydroperoxy-9ζ-octadecenoate **(8- HPOE),** 9-hydroperoxy-10ϵ-octadecenoate **(9-HPOE),** 10-hydroperoxy-8ϵ-octadecenoate **(10-HPOE)** and 11-hydroperoxy-9ζ-octadecenoate **(11-HPOE)** (see e.g. Grosch, 1987). These hydroperoxides produce 8-oxooctanoate, 9-oxononanoate, 10-oxo-8ϵ-decenoate and 11-oxo-9ζ-undecenoate, respectively, through β-scission reactions *via* route B (Scheme 2). On the other hand, the two alkenyl radicals 8-nonenoate and 9-decenoate radicals (resulting from **10-HOPE** and **11-HOPE**, respectively) produce 9-oxononanoate and 10-oxodecanoate, respectively, through β-scission reactions *via* route A.

The oxidation of linoleate yields two types of hydroperoxides, namely 9-hydroperoxy-9ϵ, 11ζ-octadecadienoate **(9-HPOD)** and 13-hydroperoxy-9ϵ,11ζ-octadecadienoate **(13-HPOD).** These hydroperoxides can generate 9-oxononanoate and 13-oxo-9ζ,13ϵ-tridecadienoate, respectively, through route B. The alkenyl 9,11-dodadecenoate radical generated from 13-HOPD through route A may result in the formation of 12-oxo-9ζ-dodecenoate.

It is well known that linoleic acid oxidizes much faster than oleic acid (at a ratio of 1:40-50 on basis of oxygen uptake) since the C-H bond strength of the allylic methylene groups is 77 Kcal/mole for oleate and 52 Kcal/mole for linoleate (Frankel, 1985b). Relevant to these facts, 9-oxononanoate is the major aldehydic ester in oxidized lipids containing linoleate as the major polyunsaturated fatty acid.

3. TOXICOLOGICAL SIGNIFICANCE

Feeding studies on rats showed that significant portions fo deep frying fats were resorbed (Oarada *et al.,* 1986, 1988) and that certain fractions exerted toxicological properties (Nolen *et al.,* 1967). Yoshida *et al.,* (1974) attributed the toxicity of oxidized oils (inactivation of rat liver enzymes) to their high carbonyl values. Similarly, Paik *et al.,* (1976) showed that mice dosed with autoxidized methyl linoleate had 100% mortality and suspected small molecular weight carbonyl compounds to be responsible for the toxicity.

Hydroperoxide*	Mechanism**	Aldehydic Ester Structure	Aldehydic Ester Name
(A) Oleate Hydroperoxides			
 8-HOPE	Route B		8-oxooctanate
 9-HOPE	Route B		9-oxononanoate
 10-HOPE	Route A		10-oxo-8ε-decenoate
	Route B		9-oxononanoate
 11-HOPE	Route A		11-oxo-9ζ-undecenoic acid
	Route B		10-oxodecanoate
(B) Linoleate Hydroperoxides			
 9-HOPD	Route B		9-oxononanoate
 13-HOPD	Route A		12-oxo-9ζ-dodecenoate
	Route B		13-oxo-9ζ,11ε-tridecadienoate

* The full names for the hydroperoxides are:
8-hydroperoxy-9z-octadecenoate (8-HPOE),
9-hydroperoxy-10e-octadecenoate (9-HPOE),
10-hydroperoxy-8e-octadecenoate (10-HPOE)
11-hydroperoxy-9z-octadecenoate (11-HPOE)
9-hydroperoxy-9e, 11z-octadecadienoate (9-HPOD)
13-hydroperoxy-9z, 11e-octadecadienoate (13-HPOD)

** For the mechanism, see scheme 1

Scheme 2
The names and structures of aldehydic acid esters and their hydoperoxide precursors

Later, the mutagenicity of deep frying fats was positively correlated with their content of thiobarbituric reactive substances (Hageman *et al.,* 1990).

Generally, aldehydic compounds are known to crosslink to the free amino functional groups of proteins forming irreversible Schiff's bases with possible serious biological implications (Maier, 1973; Maier & Hartman, 1977; Gardner, 1979, 1983). Aldehydes can also react with sulfhydryl groups of certain proteins causing functional modifications of DNA, enzymes and other cell proteins. Alpha, beta-unsaturated aldehydes (e.g. 10-oxo-8ε-decenoate and 11-oxo-9ζ-undecenoate) are the most reactive electrophiles in this context due to their high ability to function as direct-acting alkylating agents. The toxicological properties of aldehydes were attributed to their ability to inhibit protein synthesis, inhibit enzymes, block macrophage action and stimulate thrombin production *in vivo* (Tappel, 1975; Turner *et al.*, 1975, Schauenstein *et al.,* 1977; Barrowcliffe *et al.*, 1984; Van Hinsbergh, 1984; Cooper *et al.,* 1987; Witz, 1989; Novotny *et al.,* 1994).

Although some aldehydes of biological origin (mainly 4-hydroxy-*trans*-2-nonenal and malondialdehyde) were extensively studied and shown to produce a number of deleterious effects in cells, biomembranes and certain enzymes (Schauenstein & Esterbauer, 1979; Esterbauer *et. al.,* 1990), the knowledge about the absorption and toxicity of the aldehydic acids generated from peroxidized lipids is quite limited to a few studies mainly reported by Kanazawa and his co-workers. Using radiolabelled substances, it was shown that 2.6% of orally fed secondary autoxidation products of linoleic acid accumulated and induced lipid peroxidation in rat liver (Kanazawa *et al.,* 1985, 1986). 9-Oxononanoic acid and hexanal were detected in the hepatic mitochondrial and microsomal lipids (Kanazawa & Natake, 1986). Synthetic 9-oxononanoic acid also increased the lipid peroxide level as well as the activities of glutathione peroxidase and glutathione reductase in the liver (Minamote *et al.,* 1985). This aldehydic acid also inhibited the de *novo* synthesis of fatty acids by decreasing the activity of acetyl-CoA carboxylase (Minamoto *et al.,* 1988).

Kanazawa *et al.,* (1988) also studied the toxicity of orally administered linoleic acid and its autoxidation products on the intestinal mucosa in the rat. Hydroperoxides and secondary oxidation products showed different effects on intestinal microvillous membranes. Hydroperoxides polymerized the membrane proteins and led to hemmorrage and diarrhea and subsequently certain enzymes (namely sucrase, maltase and alkaline phosphatase) were leaked from the membrane and decreased in activity. The secondary oxidation products, on the other hand, coated the surface of the membrane proteins and decreased membrane permeability leading to increased enzyme activity. When hydroperoxides are ingested, low amounts are easily decomposed or reduced in the alimentary canal while large amounts are probably excreted during diarrhea (Holman & Greenberg, 1958). The secondary oxidation products, on the other hand, stay longer in the intestine (Kanazawa *et al.,* 1985) and may chronically affect the intestinal mucosa (Kanazawa *et al.,* 1988). Actually, the nutritional significance of dietary aldehydic esters derived from fried foods needs further investigations as regards short –and long– term effects on the various parts of the alimentary canal, liver and other tissues.

4. ANALYTICAL METHODOLOGY

A proper methodology for the quantitation of aldehydic acids in frying oils has not yet established. Schwartz *et al.,* (1994) published a method for the quantification of total oxo-fatty acids (OFA, aldehydic + ketonic acids) in heated fats and oils using column chromatographic separation of 2,4-dinitrophenyl hydrazone (2, 4-DNPH) derivatives followed by spectrophotometric determination. An alumina column was eluted with hexane-toluene (3:2, v/v) and two OFA fractions were collected. The first fraction, which was eluted between 50 and 75 mL, contained acids having a double bond in conjugation with the C=O bond (e.g. 10-oxo-8ϵ-decenoate and 11-oxo-9ζ-undecenoate). This fraction had a λmax of 352 nm in cyclohexane and 365 nm in $CHCl_3$. The second fraction which was eluted between 75 and 125 mL contained saturated aldehydic acids (e.g. 8-oxooctanoate and 9-oxononanoate), aldehydic acids with double bond(s) not conjugated with the C=O bond (e.g. 12-oxo 9ζ-dodecenoate) as well as ketonic acids. This fraction had a λmax of 350 nm in cyclohexane and 370 nm in $CHCl_3$. Authentic methyl 9-oxononanoate (second OFA fraction) had a λmax of 335 nm in cyclohexane and 352 nm in $CHCl_3$.

Kuksis and co-workers used the term «core aldehydes» as a generic term referring to aldehydic acids bound to lipid parent molecule such as glycerol (Kuksis, 1990; Kuksis *et al.,* 1993) or cholesterol (Kamido *et al.,* 1993). They developed methods for the analysis of these species using combinations of TLC and LC-MS. Using these methods, the core aldehydes were separated from the lipid extracts by TLC and then derivatized to 2,4-DNPH. The hyrazones were further separated by TLC into a number of bands and each band is then isolated and analyzed by reversed-phase HPLC-MS. Using this approach, it was possible to analyze cholesterol-bound aldehydic acids (Kamido *et al.,* 1993). However, when the method was applied to glycerol-bound aldehydes, the situation became very complicated due to the very large number of possible species attached to the glycerol molecule (fatty acids, peroxides, epoxides, hydroxy acids, etc.). If optimized, this approach may be useful to obtain qualitative data on the types of aldehydic acids formed, their associations with parent lipid molecules and relative positions on the glycerol molecule. Such information could be very useful for understanding the mechanisms of lipid peroxidation. Nevertheless, the method is highly complicated and have quantitative limitations if multi-fatty acid samples at different oxidation levels are to be compared.

The quantitative analysis of aldehydic acids requires a concentration step prior to the quantitation step. Kamido *et al.* (1993) separated the core aldehyde bands by TLC on silica gel H using heptane/isopropyl ether/acetic acid (60: 40: 4, by vol.) as a mobile phase and Schiff's reagent as a localization spray. Alternatively, the aldehydic acids can be separated from original lipids (or their methyl esters) by adsorption chromatographic methods (e.g. Márquez-Ruiz *et al.,* 1990; Schwartz *et al.,* 1994). Once the aldehydic acids were concentrated (together with hydroperoxides, epoxides and/or other polar compounds), they can be analyzed by GC or by HPLC as 2,4-DNPH. For the quantitation of glycerol-bound aldehydic acids, transesterification is necessary and can be performed before or after the concentration step.

Recently, a paper was published on the detection of aldehydes in thermally stressed oils and fats using high resolution (400 & 600 MHz) NMR spectroscopy (Haywood *et. al.,* 1995). The aldehydic region of the spectra (9.30-9.80 ppm) provides information on total, saturated, unsaturated and 2,4-dienal aldehydes. Although the method can not differentiate between different members in the same group, it is useful for the estimation of the total aldehydic acids and their classes in oxidized oils. No report seems yet to have been published on the use of NMR for the quantitation of the aldehydic acids in oxidized lipids.

We characterized methyl 9-oxononoate, methyl 10-oxo-8-decenoate, methyl 11-oxo-9-undecenoate and methyl 12-oxo-9-decenoate in the lipid extracts from french fries and in the used and unused frying oils (Kamal-Eldin *et al.,* 1996). The method involved saponification of lipids, elimination of the unsaponifiable matter, transmethylation and further fractionation of the methyl esters into polar and non-

polar fractions by silica column chromatography (Márquez-Ruiz *et al.,* 1990). The polar fractions, containing the aldehydic methyl esters, were then separated by gas chramatography on a medium-polarity DB-Wax capillary column (30 m x 0.25 mm i. d., 0.25 μm film, J & W Scientific, Folsom, CA, USA) and characterized by mass spectrometry (at 70 eV). In that experiment, it was not possible to quantify the aldehydic acids in the analyzed samples since the samples had very low alteration leve, 1-2% of total methyl esters (Kamal-Eldin *et al.,* 1996).

In our trials to find suitable internal standards for the quantification of the aldehydic acid methyl esters (unpublished), we prepared two saturated analogues, namely methyl 11-oxo-undecanoate and methyl 12-oxododecanoate, which are not expected to be present in frying oils. The retention times of these compounds qualifies them for use as internal standards. Another possible internal standard may be the ketonic oxy acid (6-oxo-heptanoate) which elutes at 17.41 min under the same GC conditions (Table I). In case of complex mixtures, quantification may require a mass detector operated under the selective ion monitoring mode. The characteristic fragment ions suitable for selective detection of each aldehydic acid ester are indicated in table I.

Table I
Relative Retention times and MS fragmentation ions used in the identification of the aldehydic acid methyl esters

Aldehydic Acid	Rt* (min)	Main Fragmentation Ions, m/e					
		M^+	M-31	M-32	M-43	M-32-43	Other Fragments
Aldehydic Methyl Esters in Frying Oils							
Methyl 8-oxooctanoate	20.58	172	141	140	129**	97**	87, 74, 69, 59, 55, 41
Methyl 9-oxononanoate	23.39	186	155	144	143**	111**	98, 87, 81, 74, 69, 59, 55, 41
Methyl 10-oxodecanoate	26.07	200	169	168	157**	125**	87, 83, 74, 69, 59, 55, 41
Methyl 10-oxo-8-decenoate	30.07	198	167	166**	155	123	98, 87, 83, 74, 70, 69, 55, 41
Methyl-11-oxo-9-undecenoate	32.96	212	181**	180**	169	137	98, 87, 83, 74, 70, 69, 55, 41
Methyl 12-oxo-9-dodecenoate	34.94	226	195	194**	183	151	98, 87, 83, 81, 74, 70, 69, 55, 41
Methyl-13-oxo-9,11-tridecadienoate	39.79	238	207	206**	197	165	95, 81, 74, 67, 55, 41.
Aldehydic Methyl Esters for Possible Use as Internal Standards							
Methyl 11-oxoundecanoate	28.68	214	183	182	171	139**	87, 83, 74, 69, 59, 55, 41
Methyl 12-oxododecanoate	31.18	228	197	196	186	153**	87, 83, 74, 69, 59, 55, 41

* Retention times on a DB-Wax capillary column (30 m x 0.25 mm i. d., 0.25 mm film, J & W Scientific, Folsom CA, USA) programmed as follows: 90°C (2 min); 4°C/min; 240°C (40 min).
** Characteristic ions of significant abundance.

5. PREPARATION OF ALDEHYDIC ACID STANDARDS

The saturated aldehydic acids/esters can be prepared by oxidation of fatty acid-osmium tetroxide adducts (Kamido *et al.,* 1992), vicinal dihydroxy acids/esters (e.g. 9,10-dihydroxystearate) or their corresponding epoxides with periodate. Vicinal dihydroxy fatty acids can be obtained from permanganate oxidation of suitable unsaturated acids (Wiberg & Saegebarth, 1957). The epoxide can be prepared by the reaction of unsaturated fatty acids with metachloroperbenzoic acid (D. P. schwartz, personal communication).

We prepared methyl 8-oxooctanoate, methyl 9-oxononanoate, methyl 10-oxodecanoate, methyl 11-oxoundecanoate and methyl 12-oxododecanoate from the standard monounsaturated FAME: methyl 8-eicosenoate, methyl oleate, methyl 10-nonadecenoate, methyl 11-eicosenoate and methyl 12-tridecenoate, respectively, using the osmium tetroxide method (Kamal-Eldin *et al.,* 1996). This reagent readily forms 1,2-diol-ato complexes with olefinic bonds which then undergo oxidative cleavage by periodate. Since all olefinic bonds will be cleaved by these reagents, it is not possible to prepare the unsaturated aldehydic acids by this metod. The aldehydic acid methyl esters can be purified by TLC using heptane/isopropyl ether/acetic acid (60: 40: 1, by vol.) as mobile phase and Schiff's reagent as a localization spray. It is noteworthy that these aldehydic acids have a wide range of polarity (Rf 0.24-0.36) in this TLC system.

For the preparation of 9-oxononanoate in large amounts needed for feeding studies, it is advizable to prepare the acid form because of the possibility of recrystallization from water. For this purpose, oleic acid

is oxidized to dihydroxystearic acid using potassium permanganate, and then further oxidized to pelargon aldehyde (nonanal) and 9-oxononanoic acid by potassium periodate. The two aldehydes are then separated by silica column chromatography and 9-oxononanoic acid is recrystallized from water several times before use. If the methyl (or other) ester is required, the acid should be transesterified (Minamoto *et al.,* 1985).

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