

# Riscos e Alimentos

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## Olive oil and other vegetable oils



*Pesticides in virgin olive oil: what is the risk?*

*Possible contaminants of frying oils*

*Olive Oil Adulteration*

## Possible contaminants of frying oils

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### Introduction

The increased consumption of fried and pre-fried foods has been linked with an increase in the intake of fats and oils subjected to high temperatures. This has been influenced by social, economic and technical factors, since people spend less time in preparing food.

Oils and fats are usually processed to improve the quality, stability and safety. Despite the removal of a large amount of impurities from the oil, processing can often originate new contaminants that can cause additional health hazards to those who consume these foods.

The frying process provides a faster alternative cooking method, while it improves the sensory quality of food. The growth of industries that produce these foods has led to the development of new equipment for this purpose, both industrial and domestic, in which sometimes a large amount of oil is subjected to heating for long periods of time [1]. In the conventional frying cooking method, food is immersed into the frying oil/fat at a temperature of 180 °C, which acts as the heat transfer medium. This form of heating is more efficient than cooking with hot air or cooking in water, since the temperature reached by the frying process is higher than those achieved by boiling water or by steam. Since part of the oil used for the heat transfer is absorbed by the food, thus becoming part of the food, the frying medium must have quality that needs to be maintained all over the process.

When the oil used in the frying process is heated for long periods, several complex reactions which produce a large number of compounds may occur, resulting in the degradation of the oil/fat. During these reactions, the functional, sensory and nutritional quality of the oil and the food can be modified, and it is not possible to continue to produce food with nutritional quality and safety.

An adequate intake of dietary fat is crucial for a healthy development. In addition, to the energy provided by the intake of fat, this nutrient also plays an important role with respect to our daily energy requirements and enables the absorption of fat-soluble vitamins. The recommended daily intake varies with age, health status and lifestyle of individuals. An excessive intake of fat has been linked as responsible for the increased risk of obesity, coronary heart disease and certain types of cancer. The mechanisms by which these diseases can be developed are complex and in many cases those mechanisms are still not fully understood.

### Changes of frying oils during frying

Some of the chemical reactions that are involved in the frying oils degradation are: hydrolysis, oxidation, isomerisation and polymerisation. These chemical reactions can result in the production of free fatty acids, aldehydes, ketones, diglycerides and monoglycerides, *trans* isomers, and other compounds [2]. To avoid oxidation of oils and fats it is necessary to reduce the incidence of all factors that can induce this process, such as: keeping the minimum levels of energy (temperature and light), that are responsible for triggering the formation of free radicals process, avoiding the presence of trace metals into the oil, avoiding the contact with oxygen and preventing the formation of free radicals by means of antioxidants addition [3].

During the frying cooking method, the oil/fat undergoes a complex process of degradation yielding drastic changes in its structure. Therefore, it becomes necessary to use more stable oils and fats, among which palm oil and hydrogenated vegetable oils can be highlighted. These oils have low levels of polyunsaturated fatty acids (PUFA), which as a consequence, lead to the presence of considerable amounts of saturated fatty acids (SFA) and/or *trans* fatty acids (TFA) in

fried products. Currently, the production of high stability oils with low levels of PUFA, obtained by genetic modification of oilseeds, or oils with a high content of oleic acid, whose fatty acid composition and triglyceride is very different from conventional oils, has been increased [4]. During the frying process some toxic compounds that can be produced in the used oils/fats are, for example, 3- monochloropropane-1,2-diol (3-MCPD) and 4-hydroxy-2-*trans*-nonenal (HNE).

### **Trans fatty acids**

TFA have been defined by different organizations, for instance *Codex Alimentarius* (1985) and EFSA (2010) [5, 6]. Although they are consistently the same, small differences may be found between them. According to *Codex Alimentarius*, TFA are “all the geometrical isomers of monounsaturated and polyunsaturated fatty acids having non-conjugated, interrupted by at least one methylene group, carbon-carbon double bonds in the *trans* configuration”. EFSA specifies that *trans* polyunsaturated fatty acids “have at least one *trans* double bond and may therefore also have double bonds in the *cis* configuration”.

Analytical methods are critical elements for the correct and accurate determination of TFA. Several techniques have been used to determine TFA content in foods and the most common are gas chromatography (GC) and reversed phase liquid chromatography (HPLC). In GC, either flame ionization detector (FID) or mass detector (MS) can be used, although MS detector allows a better confirmation of peak identities, not only comparison of the retention time with those of standards, but also by comparison of mass spectral information. FA have relatively high polarity, so they are generally derivatized in the corresponding fatty acid methyl esters which are non-polar derivatives [7]. With respect to chromatographic columns for TFA analyses, the most common are fused silica capillary columns of 100 m, since longer columns can reduce the risk of TFA isomers overlap [8].

There are three main sources for the origin of *trans* fats. The first source is the partial hydrogenation process which converts liquid vegetable oils into solid or semi-solid fats with appropriate melting properties suitable for products such as shortenings and margarines. The second source is the natural presence in fats from ruminant animals formed in their stomach by microbial hydrogenation of *cis*-unsaturated fatty acids. The third source of TFA is heat treatment [9].

The role of dietary fats and oils in human nutrition is one of the most complex and controversial areas of investigations in nutrition science. Dietary fat is perceived to be the

“worst” of all the nutrients in promoting various diseases, like cardiovascular disease (CVD), diabetes, obesity, and certain types of cancers. According to the literature, TFA are associated with an undesirable effect on serum lipid profiles, and thus may increase the risk of CVD, being considered in this respect, worse than saturated fat [10]. A daily intake of 5 g of TFA was associated with an increase of 25% in the risk of coronary heart disease [11].

Over the years, several studies have been conducted in many countries to determine the levels of TFA in foods produced by industries, bakeries and fast food chains, with the objective of identifying the various food sources and to estimate the daily intake of TFA. According to Larqué et al. (2001), foods that contain partially hydrogenated fats contribute about 80% to 90% of the daily intake of TFA [12]. With respect to animal fats this contribution is much smaller, estimated at 2% to 8%. Refined oils have reasonably low TFA levels (1.0-1.5%), but the neutralization, especially in the preparation of fried foods, can make a significant contribution to the daily intake of TFA [13]. However, TFA are also produced during the preparation of margarines, when the PUFA from liquid oils are artificially hydrogenated to produce solid fats. Significant amounts of TFA are found in margarines, butters and some other types of industrial products that contain hydrogenated fat [14].

In Portugal, few studies in the last years, have determined TFA levels present in oils/fats. However, over the 90's, some studies were carried out by Portuguese researchers regarding the levels of TFA in various food groups, and some of the published results for margarines, oils and butters, are presented in Table 1.

**Table 1.** TFA methyl esters content (g/100 g of fat) in margarines, oils, butters and shortenings.

Foods	TFA <sup>a</sup> (g/100 g of fat)
Table margarines	0.23 – 14.8
Culinary margarines	0.95 – 13.1
Industrial margarines	3.15 – 12.9
“Shortenings”	0.07 – 16.9
Liquid fat for culinary	0.13
Butter	4.62 – 5.26
Vegetable oils	0.13 – 1.55
Cooking oils	0.14 – 0.25

Adapted from [15]

<sup>a</sup> The values are presented in g of fatty acids methyl esters per 100 g of fat.

In addition to the determination of fatty acids content in raw oils/fats, studies were also performed to assess the fatty acids composition of oils submitted to continuous frying operations [16]. In this study, frying tests were conducted in an oven up to 96h (using only the oil) to evaluate the formation of TFA in oils/fats that are commonly used in the frying process. Furthermore, the fatty acid profile of the oils used to fry potato chips, octopus fillets and meat rissoles was determined, as well as the fatty acid composition of the fried foods. It was concluded that the presence of TFA is dependent on the nature of the oil, i.e. soybean oil showed the highest levels compared to the frying oil. Nonetheless, frying oil showed more stability to frying. Moreover, it was also concluded that the fatty acid composition and the *trans* isomers of the frying bath, is a crucial factor to the final fat composition of the fried product [16].

### 3-monochloropropane-1,2-diol

3-MCPD is a food processing contaminant that belongs to a group of chemicals known as chloropropanols. These are chemical derivatives of glycerol, structurally characterized by the presence of one or two chlorine atoms. According to scientific literature, 3-MCPD is found in greater abundance in food, followed by 2-monochloro-1,2-diol (2-MCPD).

In recent years, there has been an increase in the number of studies related to the development of analytical methods for determining 3-MCPD levels in foodstuffs, as demonstrated by the recent scientific review of Crews et al. (2013) [17]. The analysis of these contaminants is extremely complex and there are two types of methods, direct and indirect. Direct methods allow the individual identification of esters of 3-MCPD. Usually these methods are preceded by solid phase extraction and determination of 3-MCPD is subsequently performed using GC methods mainly coupled to MS detector [18]. However, methods of high efficiency liquid chromatography coupled to mass spectrometry (LC-MS), using detectors as time of flight (TOF), orbitrap and triple quadrupole (MS/MS) have also been developed [19]. In indirect methods, the total concentration of 3-MCPD esters is measured as free 3-MCPD and, most of the times, it includes the addition of an internal standard, hydrolysis, neutralization, removal of fatty acids methyl esters, and finally the derivatized 3-MCPD is analyzed by GC/MS.

The International Agency for Research on Cancer has classified 3-MCPD as a possible human carcinogen (group 2B)

[20]. There are few studies in the scientific literature, and many of them are controversial with regard to the effects of these contaminants on human health. However, in 2001, the "Scientific Committee on Food" concluded that 3-MCPD is a non-genotoxic carcinogen and established a tolerable daily intake for 3-MCPD of 2 µg/kg body weight [21]. The European Commission has established maximum levels of 20 µg/kg (for the liquid product containing 40% dry matter, corresponding to a maximum level of 50 µg/kg in the dry matter) for the occurrence of 3-MCPD in hydrolysed vegetable protein and soy sauce [22].

In the early 80's, the presence of 3-MCPD was detected in hydrolysed vegetable protein in soy sauce and similar products, which was formed as a product of the reaction of hydrochloric acid with triacylglycerols, phospholipids and glycerol present in vegetable oils [23]. Afterwards, it was found that 3-MCPD can also be present in other products which are thermally processed, such as pastry, malt-derived, smoked and/or cured fish or meat. In 2006, Zelinková et al. published the first study on the presence of 3-MCPD esters in fats and oils [24]. In Portugal, up to now, information regarding the content of this contaminant in food is still very limited or inexistent. In Table 2, some results from other countries concerning the levels of 3-MCPD in oils and fats are shown.

**Table 2.** Levels of 3-MCPD (µg/kg) in oils and fats.

Food	Range (µg/kg)	Reference
Refined olive oil	<300 – 2462	[24]
Milk fat, lard and poultry fat	<100 – 300	[25]
Margarine	400 – 4500	[25]
Frying oils (fresh and used)	500 – 5200	[25]
Refined vegetable oils	<300 – 1234	[24]
Refined vegetable oils	150 – 1880	[25]
Refined sunflower oil	100 – 2100	[26]
Refined salmon oil	700 – 13000	[26]
Refined palm oil	1100 – 10000	[26]

The quantification of the levels of these compounds in oils and fats is particularly important with respect to human health, since in addition to the oils/fats that contain very substantial amounts of 3-MCPD, these same oils/fats are often ingredients in processed foods. For example, palm oil

is commonly used in preparing foods for infants, pastry products and mayonnaise, among others. In recent years, significant improvements have been obtained regarding the development of analytical methods for determination of 3-MCPD, studies related with the formation mechanisms of these contaminants in foodstuffs, identification of new food sources, assessment of potential toxicity and development of processing techniques that minimize the formation of 3-MCPD and other chloropropanols.

#### 4-hydroxy-2-trans-nonenal

HNE is a secondary lipid peroxidation product of linoleic acid and other omega-6 fatty acids [27, 28]. It is a toxic compound and has been related to atherosclerosis, low density lipoproteins oxidation, stroke, Parkinson's, Alzheimer's and Huntington's diseases, among others [29-31].

These aldehydes are formed as a result of degradation of the fatty acids in the presence of oxygen. When vegetable oils, particularly those that are rich in polyunsaturated fatty acids, are subjected to high temperatures, which occur with frying, the risk of formation of secondary products such as HNE is increased. Due to the toxicity of HNE, which can be absorbed by food, information regarding the mechanisms of formation of this compound, particularly in fats and oils subjected to high temperatures, is very important from the point of view of public health.

The analytical determination of HNE is mainly carried out using LC-MS or GC-MS techniques [32]. For GC-MS analyses, the sample requires derivatization, e.g. using pentafluorobenzil oxime, followed by silylation, and HNE may be detected with negative chemical ionization and quantified by comparison to the internal standard (deuterated HNE) [33, 34]. Methods that involve analysis by LC-MS have the advantage of not requiring derivatization, and thus the sample may be analyzed with less extraction intermediate steps [35, 36].

Boskou et al. (2006) reported results for HNE content in oils (sunflower, palm oil and vegetable oil) used to fry potatoes, as well as, for the fried foods, and found out that the presence of HNE is mainly related to the type of oil used for frying, and not so much with thermal deterioration of the oil [37]. In 2008, Han and Csallany compared butter and vegetable oils subjected to high temperatures for short periods of time, and subjected to lower temperatures but for longer periods of time [38]. The results were similar, and once

more the fatty acid composition of vegetable oils is more important than the exposure time to the heat treatment itself, concluding that for oils/fats containing high levels of linoleic acid, the heat treatment must be performed using low temperatures to prevent the formation of HNE [38].

Scientific studies showed that HNE may be present in other foods than oils/fats of vegetable origin, like ham, bacon, and smoked sausages with varying contents between 3.77 and 95.2 mol/kg [39]. Csallany and Han (2012) have determined the levels of HNE in natural and imitation of Mozzarella cheeses, which were exposed to different temperatures and different heating times [40]. According to the obtained results, the formation of HNE was significantly lower in natural Mozzarella cheeses, which contains milk fat, in comparison with the imitation Mozzarella cheese that uses vegetable oils (high in linoleic acid) [40].

Therefore, since HNE is a toxic compound associated with negative effects on human health, more scientific research focusing on the determination of the content of this compound in other foods is needed, and the study of changes during processing that may lead to a decrease of its formation.

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