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Thermal treatment: evaluation of thermoxidative degradation in frying oils and determination of aromatic compounds in complex food matrices

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Esame finale anno 2012

To Mom and Dad

To Federica with love

For myself

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INTRODUCTION

Cooking food causes many chemical changes, physical and organoleptic properties in food, the majority of whom are desired, but others, leading to unwanted and concentration of the food to water loss, increased digestibility, due to dependent processes of hydrolysis of proteins and polysaccharides, to improve the organoleptic characteristics of attractiveness and health qualities, for the destruction of microorganisms, inactivation of enzymes and any toxic substances, the decrease of nutritional value, mainly due loss of certain amino acids (eg lysine), following the Maillard reaction and the loss of heat-labile vitamins and minerals by dissolution, hydrolysis of triglycerides, followed by successive transformations of the dependent glycerol and fatty acids. The modifications depend essentially determined by the cooking technique used, the type of product, duration of treatment and from the container used. The food can be cooked in many ways, which differ in the means of propagation and for the duration of the heat, in view of the final product that you want to achieve. Cooking techniques are numerous, among them, like the one in the oven, steaming and frying.

2.1 FOOD FRYING

The frying is among the cooking methods, the most simple and basic, which has its roots in times very remote. In fact, in the third book of the Old Testament, a distinction is made between food cooked with oil and grilled in a pan, Pliny, the great Latin writer, describes in one of his works, fried eggs (Morton, 1998). Nowadays the economics of commercial frying oil immersion is estimated at 83 billion dollars in the U.S., and at least twice the amount for the rest of the world (Pedreschi et al., 2005). This popular technique, considered by many an art more than a real science (Grob., 1990), apparently not very complex, consists of a series of physico-chemical reactions that see the progressive dehydration of the food to 'inside an oil bath maintained at high temperature (170°C-200°C) (Gertz et al., 2000) and following transfer of thermal energy between the oil bath and food itself (Baumann et al., 1995). Frying is a particular way of cooking food because it takes oil as heat transfer fluid. It, as a technique for cooking, has acquired a certain importance in the modern because it easy to use, fast and inexpensive. This cooking mode is normally used, both industrial and domestic, to impart particular organoleptic characteristic like color, aroma and texture which are able to transfer to different food. Moreover, its important to note the effect that the conservative fried food produces, as a results of thermal destruction of microorganism and the reduction of water activity on the surface of the products.

Many fried foods can be stored at low temperature (-18°C) in the form of frozen and / or frozen for varying periods of time, but usually up to a maximum of about 12 months and this is made possible because the shelf life of a lot of fried foods is partly determined by their moisture content after cooking (Barbanti, 1993). For this reason, the use of this method of preparation of food is increasing, because the unique sensory properties are accompanied by undesirable changes in the frying medium that produce new compounds that are then absorbed from food. The study and understanding of this process must therefore take into account all aspects related to the quality of the products of the oils used in frying.

The methods commonly used for frying can be traced to two standard modes: fried in thin layer of oil and fry for total immersion. The fried in a thin layer of oil (cooking plate) is possible trough a mechanism of simultaneous conduction and convection, using as an element of contact with the heating body fat and/or oil, which also become ingredients of the product. This process is particularly suitable for cooking foods with a large developed area and limited thickness (es. Burgers, bacon..). The surfaces irregularities affect food distribution of the layer of fat or oil in contact with the heating body, and this, together with the simultaneous presence of steam escaping from the food, due to temperature variations in the exchange surface, producing an uneven brewing in outermost layer of the product, characteristic of the food cooked by contact. The one most widely used industrially, in catering and also in domestic practice, is undoubtedly the immersion frying, and is also the one that is of interest both in research and in industrial applications. The immersion frying in oil (deep-fat frying) is a process of cooking food and the most widely used is a traditional fried food because they are characterized by a particularly tasty flavor, golden color and a crispy surface.

The frying to complete immersion have high temperatures (150°C - 190°C). The simultaneous heat and mass transfer between oil, food and air during the frying process produces fried attractive and unique quality. The oil used in frying acts as a heat transfer medium and contributes to the texture and aroma of these foods. The frying time, the surface area, the moisture content of foods, types of breading and frying oil affect the quantity of oil absorbed by foods (Moreira et al., 1997).

Under optimal conditions of temperature and cooking time, fried foods have a golden brown color, are crisp and well cooked and have an optimal absorption of oil (Blumenthal, 1991). Under conditions of low temperatures or short cooking times, they have, instead, a slightly brown surface color or white, and the heart is not cooked with the presence of starch gel. They have also appealing flavor and crunchy texture. Foods fried at high temperatures and for long periods show, finally, a dark surface and hardened, and an oily consistency caused by the excessive absorption of oil.

The quantity of oil in fried foods can vary, depending on whether it's potato chips and/or corn (from 30% to 38%), tortillas (from 23% to 30%), donuts (from 20% to 25 %), and noddle fried potato sticks (from 10% to 15%). The absorbed oil has a tendency to accumulate on the surface of the fried food during frying, and to move gradually towards the heart during cooling (Moreira et al., 1997).

2.2 TYPES OF FRYING

As part of the immersion frying is possible to distinguish three different types of processes: industrial, catering and domestic frying.

The industrial frying, fried products used for prolonged shelf life (snacks), is a continuous process in which food is dipped in vegetable oil of average quality in most palm oil. In this type of frying oil is not replaced frequently, but simply refilled. The topping is the recommended daily from 15% to 25% capacity of the fryer (Stevenson et al., 1984). The catering frying food method is a semi-continuous process in which they are alternating periods of work with periods of rest of the oil. In this type of frying oil is usually replaced at least once a week.

The domestic frying is a batch process which involves the use of small amounts of oil (1-2 L), usually removed immediately after the process (Parisini, 2006).

2.3 FRYING TYPES

Frying food can be considered a process of dehydration of foods. In this process, the food is immersed in a bath of hot oil held at elevated temperatures of 180°C in air for a period of time variable (Alvis et al., 2009). Under these conditions we observe a succession of reactions, such as:

- ✓ reduce oil temperature during the immersion of the food oil for frying;

- ✓ changes to the detriment of components in the food, such as protein denaturation, starch gelatinization, browning nonenzymatic (Maillard), hydrolysis and oxidation of lipids, formation of acrylamide, etc.;
- ✓ formation of vapor contained in the food from the water;
- ✓ dehydration of the surface of the food with the formation of crust;
- ✓ consumption of oil by the food itself;
- ✓ hydrolysis and therm oil for frying (Fritsch, 1981).
- ✓ The crust is golden in color and darkness as it is subjected to processes of roasting, caramelization and non-enzymatic browning reactions (Maillard reaction), giving a pleasing appearance and at the same time improve the sensory quality of the product, making it tastier. Are preferable, in fact, foods rich in starch and protein, while the poor food of these components require breading or batter with flour, are an example potatoes, bananas, tapioca, cassava. The advantage of the frying process compared to other processes lies in the rapid heating and uniformity of the finished product because the entire surface of the product is subjected to the same heat treatment. During frying, the thermo-physical properties of the product undergo a gradual change. There is a reduction in the density of the food and the pores. The thermal conductivity decreases as the porosity increases, the specific heat, however, decreases with the reduction of moisture content and increases with the content of oil during frying.
- ✓ Immersion frying oil can be divided into four steps (Farkas et al., 1996):
- ✓ 1. Initial heating;
- ✓ 2. Surface boiling;
- ✓ 3. Falling;
- ✓ 4. Bubble end point.

In the first step, the surface of the food immersed in an oil bath reaches a temperature close to the boiling point of water. The heat transfer between oil and food is a natural convection and that there is no evaporation from the surface.

In the second step, the water starts to evaporate from the surface of the food, after the temperature reaches the boiling point value. At this point the heat transfer by natural convection is no longer forced to become as a result of turbulence that are created in the oil surrounding the food.

In the third step, the internal temperature of food is gradually increases until it reaches the boiling point, due to the presence of internal moisture. E 'in this phase that may occur, chemical and physical changes, such as gelatinization and denaturation of proteins, increased thickness of the crust on the surface and decreases the transmission of water.

In the final step, the rate of elimination of moisture decreases and no longer observe the bubbles on the surface of the product.

These four steps can be summarized into two-phase non-boiling (first and fourth step) and boiling phase (second and third step). The first requirement is the quality of the food security of its components. This is achieved through preventive risk analysis and checking the frying process, so in order to obtain better quality fried foods and be able to control the process, you need to know in immersion frying oil transfer coefficient Thermal convection considering the effects of evaporation. The coefficient of heat transfer during boiling, plays a key role in the formation of sensory properties and characteristics of the product is responsible for the golden color (Maillard reaction) and reaction caramelisation, which together contribute to the flavor, color and texture. It characterizes the flow of heat through the interface liquid/solid. Therefore, the determination of the coefficient of heat transfer by convention plays an important role in understanding the complex system of frying.

2.3.1 HEAT AND MASS TRANSFER

A generic process of cooking is characterized by a dominant transport mechanism, which directly affects the quality of the product. Specifically, during the frying oil immersion is the transport of heat (oil product), the transport of matter (water, oil from the product), occur simultaneously. The magnitude of the changes that take place depend on different chemical and physical parameters such as temperature, heating time, type of frying oil and food, mixing oil treatments on the oil and finally the equipment used (Alvis et at., 2009). The water also appears to be important because during frying, evaporating, preventing food to overcome the temperature of 100°C, avoiding a possible charring of the food itself that may occur at the temperatures of the bath of action 'oil (Blumenthal, 1991). The water also, as a constituent of the food, regulates the process of conduction, as an effective heat conductor (Orthoefer et al., 1996). During the process of frying food energy transfer occurs from the source of heat with oil, and from this the surface of the food in it surrounded by convective motions and finally, from the surface of the food inside by conduction (Parisini, 2006). While there are three distinct mechanisms of heat transfer (conduction, convention and radiation), in the case of the frying process are prevalent conduction (heat transfer within the product) and convection (heating oil and convective motion) (Barbanti, 1993).

2.3.2 CONDUCTION

The heat transfer occurs between adjacent molecules or particles, without moving in space of the molecules themselves, as is done in the case of solid foods. Assuming stationary conditions, ie that the temperature gradient between heat source and heated body is constant in time and therefore the heat flux produced for a flat surface can be considered the Fourier equation in integrated form:

$$Q = -KA\frac{(T1 - T2)}{S}$$

where:

Q = heat flow (W);

K = coefficient (average) thermal conductivity characteristic of the product (Watts / (meter * Kelvin));

A = thickness (m2);

S = surface area (m);

(T1 - T2) = temperature gradient with T1> T2.

In this case, given a mean value of the coefficient of thermal conductivity, since it is this parameter varies with temperature.

2.3.3 CONVENTION

The heat transfer by convection occurs as a result of mixing of fluids at different temperatures. The agreement is natural in this case because temperature gradients cause changes in the fluid density and thus mixing of the fluid itself. The heat transfer by convection is expressed by the relation (integrated):

$$Q = UA(T1 - T2)$$

where:

Q = heat flow (W);

U = overall heat transfer coefficient (W / (m^2 * Kelvin));

A = area (m2);

(T1-T2) = temperature gradient between the portions of fluid between the fluid and wall, or with T1> T2.

The overall heat transfer coefficient, also called laminar coefficient, takes into account various parameters that influence the convective process, such as density, viscosity and specific heat of the fluid and the presence of laminar flow can affect the wall heat transfer.

The immersion of a food in the cold bath of frying causes an immediate lowering of process temperature, due to evaporation of water from the surface of the product. The formation of steam from water requires, in fact, thermal energy is supplied from the oil environment (Blumenthal, 1991). The plan for the evaporation of water moves gradually toward the center of the food and the outer part of the product begins to undergo a gradual drying, similar to what occurs in other cooking operations, with the formation of surface crust. Consequently, the surface temperature of the product increases, tending to the value of oil temperature, while the interior of the food slowly reaches values close to the temperature of boiling water. The rate of heat transfer is controlled by the temperature gradient between the oil and product, and the coefficient of heat transfer on the surface of the product itself, the rate of heat transfer in the product is controlled by the typical thermal conductivity of the material in question. The thickness of the interface oil-product controls the rate of heat transfer and material, and is in turn determined by the viscosity and the speed of movement of the oil. The vapor pressure gradient between the inside of the product and the oil is the driving force for moisture loss.

2.3.4 MASS TRANSPORT

The mass transport, as in the case of a product during frying is done both from the surface of the food inside. Fick's law governs these processes.

For the transport of matter outside (without resistance), the relationship can be described as:

$$Fe = \frac{m\,Kc\,(P1 - P2)}{RT}$$

where:

Fe = external flow of matter (g/cm2sec);

m = molecular weight;

Kc = coefficient of matter transfer (cm / sec);

(P1-P2) with P1 = pressure gradient> P2;

R = gas constant (J / ° K mole);

T = absolute temperature (° K).

Inside the product flow of matter is governed according to the report:

$$Fi = \frac{m\,D(P1 - P2)}{RT(x)}$$

where:

Fi = flow of matter inside (g/cm2sec);

m = molecular weight;

D = diffusivity (cm2 / s);

(P1-P2) with P1 = pressure gradient> P2;

R = gas constant (J / ° K mole);

T = absolute temperature (° K);

X = thickness to be traversed (cm).

During frying, the heat is transferred to the food and water on its surface reaches its boiling point with the formation of steam. The oil temperature is decreased after the addition of the food and the

internal temperature increases slowly, remaining at around 100°C, while the water flows from the inner to outer surface. The steam limits, therefore, the absorption of the oil across the surface, getting a fried product in which you can define two distinct zones: the surface of dehydrated, over which major changes occur, and the interior of the food or the heart where the temperature exceeds 100°C. The loss of water and oil absorption in a given food are interrelated as well as the internal pressure is greater than the outside food and this limits the penetration of oil for frying. The main parameters that determine the absorption of oil or water loss are temperature and time. The temperature should not have a significant effect between 150°C and 180°C although, in general, higher temperatures correspond to a reduced absorption of oil from the surface and excessive absorption of oil would result from the low-temperature frying. Other parameters affect properties such as the shape of the food itself, the composition or the modifications undergone by the product during the treatment (Dobarganes et al., 2000).

The complexity of this process is due both to the contemporary verification of mass and heat transfer between the food and the means of frying it to the various chemical reactions that affect the oil on the one hand, the other the food fried in it. These, in fact, undergo several changes such as gelatinization, the Maillard reaction, denaturation of proteins and the drop in humidity (Parkash & Gertz, 2004). The consequence of these changes are the dehydration of the surface layer, formation of the crust and the passage of steam through the food (Moreira et al. 1999; Parkash & Gertz, 2004).

2.4 FRYING CHEMISTRY

Deep-frying is a complex process that involves food immersion in an oil bath kept at high temperatures (between 160°C and 180°C), in contact with air (Gertz, 2000). The fat involved undergo a large number of changes that affect a large spectrum of chemical reactions. Hydrolysis, polymerization and thermoxidation are the main reactions that occur during frying which effectively

leads to gradual decrease of the initial oil quality and causes changes in the physical and chemical properties of frying bat (Gertz, 2000). The quality (nutritional and organoleptic) of the oil are gradually fail as a result of prolonged heating and in particular by the presence of oxygen and water released from food. More than 500 different chemical compounds formed as a results of autoxidation, thermoxidation, pyrolysis and polymerisation have been detected in used frying oils (Gertz, 2004). These modifications can generate a series of chemical compounds with adverse nutritional implications and potentially hazardous effects for human health (Marquez-Ruiz et al., 1996). Thermoxidation, hydrolysis, and polymerization are the main reactions that occur during frying, which induce a faster decrease of the initial oil quality.

2.4.1 LIPID OXIDATION

When a fatty substance is in contact for some time with oxygen, some macroscopic phenomena that indicate the presence of a degradation in place. Looking at the phenomena more closely and analyzing their causes, four factors appear more 'other influence the trend:

• presence of oxygen;

• unsaturation of the fat;

• presence of metals;

• heat and radiation, especially ultraviolet light in the presence of sensitizers (chlorophyll, phaeophytins, hemoglobin, ematoporfirine, etc..).

The reactivity of lipid oxidation systems is, in particular, greatly influenced by unsaturation degree. After the critical point, as you indicated in Figure 2.2, the reaction proceeds faster. The first period is called induction, the second propagation.

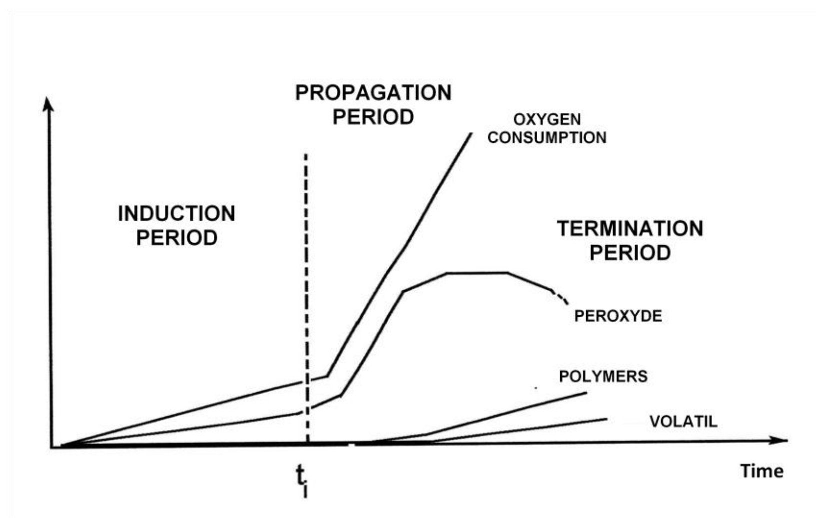

Figure 2.2 Trends of major lipid oxidation derivatives, observed as a function of time

The oxidation process proceeds via a free radical mechanism of chain reactions, where RH• represents here the triacylglycerol molecule undergoing oxidation in one of its unsaturated fatty acyl groups. In the initiation stage, an alkyl radical is formed by abstraction of a hydrogen radical from an allylic or bis allylic position of an unsaturated fatty acid. In the propagation step, the alkyl radical reacts with oxygen at rates controlled by diffusion to form peroxyl radicals that in turn react with new triacylglycerol molecules giving rise to hydroperoxides as the primary oxidation products and new alkyl radicals that propagate the reaction chain. Finally, in the termination stage, radicals react between them to yield relatively stable non-radical species.

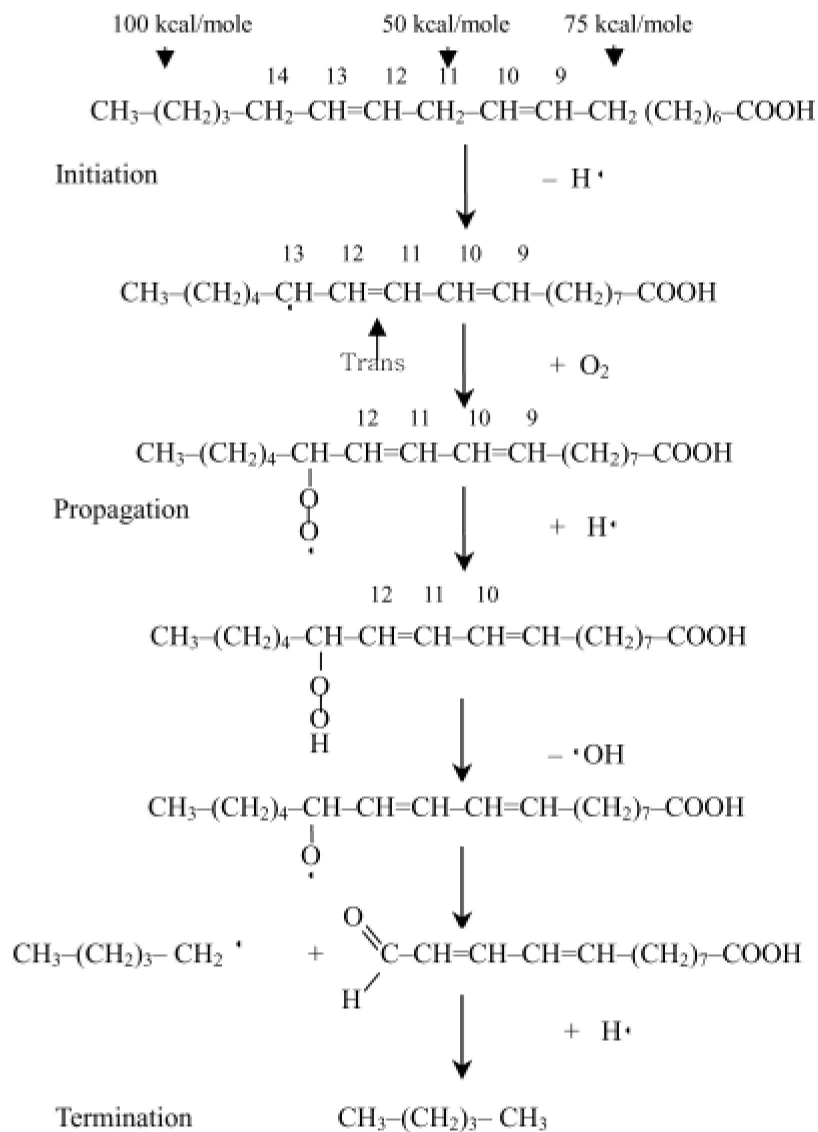

Figure 2.3 The initiation, propagation, and termination of thermal oxidation of oil (adapted from Choe and Min, 2007)

2.4.2 OXIDATIVE REACTION IN FRYING

References for this section: *Joaquín Velasco, Susana Marmesat, and M. Carmen Dobarganes*

The chemistry of lipid oxidation at the high temperatures of food processes like baking and frying is highly complex because oxidative and thermal reactions are involved simultaneously. As temperature increases, the solubility of oxygen decreases drastically, although all oxidation

reactions are accelerated. The oxidation process is proceeds via a free-radical mechanism of chain reactions, where RH represents the TG molecule undergoing oxidation in one of its unsaturated fatty acyl groups. At frying temperature, as the oxygen pressure is reduced, the initiation reaction becomes more important and the concentration of alkyl radicals (R•) increases with respect to alkylperoxyl radicals (ROO•). As a result, polymeric compounds are predominantly formed through reactions mainly involving R• and alkoxyl radicals (RO•) (Scott 1965). These facts are in agreement with the experimental results stated below.

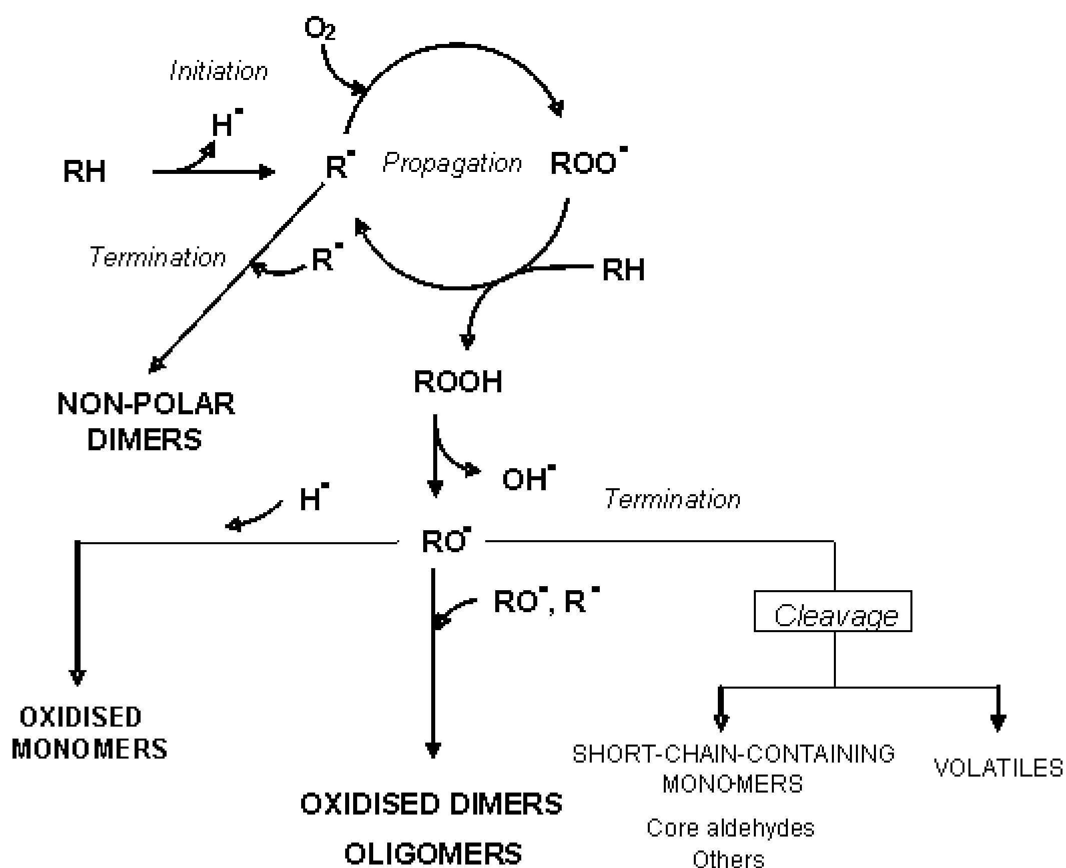

Figure 2.4 Mechanism of thermal oxidation (schematic).

At low or moderate temperatures, formation of oxidation compounds during the induction period is slow; hydroperoxides (ROOH) are the major compounds formed, and their concentration increases until the advanced stages of oxidation. Polymerization compounds become significant only in the accelerated stage of oxidation after the end of the induction period (Marquez-Ruiz, Martin-Polvillo,

and Dobarganes, 1996). However, the minor volatile compounds formed, in particular carbonyl compounds, are of enormous sensory significance, and may contribute to negatively modify oil flavor (Forss, 1972; Frankel, 2005). At high temperatures, formation of new compounds is very rapid. ROOH are practically absent above 150°C, indicating that the rate of ROOH decomposition becomes higher than that of their formation, and polymeric compounds are formed from the very early stages of heating (Dobarganes, 1998). In addition, formation of significant amounts of non-polar TG dimers (R–R), typical compounds formed in the absence of oxygen through interaction of R•, is a clear indication of the low oxygen concentration (Dobarganes and Perez-Camino, 1987). In summary, two TG radicals, R• formed in the initiation reaction and RO• formed by ROOH decomposition, are involved in the set of termination reactions. This leads to various products of different polarity, stability and molecular weight. Three main groups of compounds can be distinguished by molecular weight, as detailed below. TG dimers and oligomers are the most specific compounds in used frying fats, and are formed through interaction between TG radicals. Their molecular weights are higher than those of RH. Oxidized TG monomers are TG with at least one of their fatty acyl groups oxidized. They are formed through interaction between TG radicals and hydrogen or hydroxyl radicals. Their molecular weight is similar to the parent non-altered TG (RH). Volatile compounds are formed through breakdown of RO• and have molecular weights lower than those of RH. As previously stated, these reactions take place in the unsaturated fatty acyl groups attached to the glyceridic backbone and, therefore, the stable final products are TG monomers, dimers and oligomers containing modified and non-modified acyl groups.

2.4.3 TRYGLICERIDE DIMERS AND OLIGOMERS

References for this section: *Joaquín Velasco, Susana Marmesat, and M. Carmen Dobarganes*

The development of polymerization reactions accounts for the major and most complex group of degradation products found in used frying fats and oils. The complexity results from the different

possibilities of oxidation of the unsaturated fatty acids, along with the composition of the fat with a high proportion of TG containing more than one unsaturated acyl group per molecule. It also explains the lack of studies on the structure and formation of TG dimers and oligomers. Significant information on the mechanisms of polymerization reactions has been limited to the formation of the dimers obtained in the first step of polymerization. The studies were carried out starting from fatty acid methyl esters (FAME) be ate under well-defined conditions in the absence or presence of air. When heating in the absence of air, the compounds obtained have no extra oxygen, and will be considered separately in the next section on thermal reactions.

The structures of the compounds containing extra oxygenated functions are still largely unknown. Difficulties are due to the heterogeneity in this group of compounds. First, different oxygenated functions are likely to be present in the dimeric linkage or in any other unsaturated fatty acyl group of the molecule. Second, more than one functional group can be present in the same dimeric molecule. Therefore, the large number of possible combinations results in a very complex mixture that is difficult to separate. Under these conditions, studies have paid more attention to the composition of alteration products than to the mechanisms involved in dimer formation.

The basic knowledge on polar dimers has been obtained by heating FAME, TG or fats and oils in air, or by thermal decomposition of FAME hydroperoxides. Among the studies carried out, those giving information on the dimers found in used frying fats and oils or heated under simulated frying conditions are especially interesting (Christopoulou and Perkins 1989a, 1989b, 1989c, 1989d). After separating fractions of different polarity by adsorption chromatography, identification techniques, including mass spectrometry, nuclear magnetic resonance and infrared spectroscopy, have been helpful in providing evidence of some of the dimeric structures formed. The more noteworthy results are outlined below. Interestingly, the occurrence of dehydrodimers, bicyclic, tricyclic and Diels Alder non-polar dimers has been reported by different authors (Christopoulou and Perkins 1989a; Ottaviani et al. 1979), suggesting the significance of the allyl radical and formation of non-polar conjugated dienes, even in the presence of oxygen. Among products bearing

oxygenated functions, acyclic dimers with C–O–C linkages and cyclic dimers such as tetrasubstituted tetrahydrofurans have been isolated from low-polarity fractions obtained after transesterification of heated soybean oil (Ottaviani et al. 1979). Structures found for polar dimers were mainly C–C linked dimers containing monohydroxy, dihydroxy and keto groups (Christopoulou and Perkins 1989a). Structures for compounds with molecular weight higher than that of dimers have not been reported in methyl esters from frying fats or in model systems. This is not unusual considering that much more research remains to be done on structure elucidation and quantification of simpler molecules, i.e., oxidized monomers and dimers, which are intermediates in the formation of trimers and higher oligomers.

2.4.4 OXIDIZED TRIGLYCERIDE MONOMERS

References for this section: *Joaquín Velasco, Susana Marmesat, and M. Carmen Dobarganes*

Oxidized TG monomers are characterized by at least one extra oxygen atom in at least one of the three fatty acyl chains. They are stable final products resulting from the decomposition of primary oxidation compounds (ROOH). The main functional groups present in frying oils correspond to epoxy, keto, and hydroxy. The formation of the epoxide ring has been reported to proceed through the reaction of a double bond with an external ROOH (Giuffrida et al. 2004). This mechanism explains the presence of the ring at the site of a double bond, and the concomitant formation of the hydroxy function from the corresponding ROOH. As for the hydroxy and keto fatty acyl groups, information is limited due to the complexity provided by structures with a higher number of double bonds, which makes their separation difficult. As degradation progresses, more than one oxygenated function may even be present in the same fatty acyl chain, and more than one oxidized fatty acyl group may be present in one TG molecule. One important subgroup among the oxidized fatty acyl groups are those originated by ROOH breakdown (Kamal-Eldin et al. 1997). The main mechanism for aldehyde formation from lipid hydroperoxides through homolytic scission of the C–

C bonds on either side of the RO•. This cleavage results in two types of aldehydes: volatiles derived from the methyl side of the fatty acid chain, and aldehydes still bound to the TG. The volatile aldehydes have special interest for their contribution to the sensory properties of used frying fats and oils. The formation of non-volatile aldehydes is of enormous chemical and nutritional interest in deep frying because they remain in the oil, are absorbed by the food, and are subsequently ingested. However, their quantitative importance is low compared with other groups of oxidized monomers.

2.4.5 THERMAL REACTIONS

References for this section: *Joaquín Velasco, Susana Marmesat, and M. Carmen Dobarganes*

Thermal reactions are those taking place without the participation of oxygen. They give rise to products of low polarity, i.e., TG without extra oxygen in the molecule. Even though the frying process takes place in air, many non-polar degradation products have been identified in used frying fats due to the low availability of oxygen at high temperature. The main groups of compounds found are TG dimers or isomeric TG, including cyclic or *trans* fatty acyl groups. The mechanisms of reaction involved in the formation of non-polar dimers, i.e., products bearing C-C linkages and without extra oxygen in the molecule have been studied by using FAME subjected to high temperatures, usually between 200 and 300°C, in the absence of air to inhibit oxidative reactions. Analysis by mass spectrometry of dimers isolated before and after hydrogenation of samples gave clear information on the rings and the number of double bonds present in the original structures, as well as on isomeric forms. From a large series of experiments, the general conclusions detailed below stand out:

The major compounds identified during thermal treatment of FAME are generated through radical reactions from the allyl radicals and, consequently, the oxidation pathway is involved in their formation (Figge 1971). In the presence of conjugated double bonds, thermal dimers originate after

a Diels Alder reaction. Thus, a tetrasubstituted-cyclohexene structure is formed from the reaction of a double bond of a molecule, acting as dienophile, with a conjugated diene of another one (Sen Gupta and Scharmann, 1968). The proportion of different dimers depends on the conditions. For example, at 140°C, only dehydrodimers were found in significant amounts. Conversely, above 250°C, mono-, bi-, and tricyclic dimers were mainly detected, whereas dehydrodimers were not (Sen Gupta, 1969). Even though thermal conditions in the absence of air are far different from those used in frying, the techniques applied in these studies have been of great help in the identification and quantifi cation of non-polar dimers in used frying fats. The levels found indicate that non-polar dimers may be one of the most relevant groups of new compounds formed during frying (Marquez-Ruiz, Perez-Camino, and Dobarganes 1990; Marquez-Ruiz, Tasioula-Margari, and Dobarganes 1995). However, the presence of non-polar oligomers in used frying fats has not been reported.

2.4.6 HYDROLYSIS

References for this section: *Joaquín Velasco, Susana Marmesat, and M. Carmen Dobarganes*

Hydrolysis is the well-known reaction affecting fats and oils due to the action of lipolitic enzymes or moisture. In frying, the reaction is of great interest due to the: high content of moisture of most foods subjected to frying. Technological problems originated by the formation of free fatty acids (i.e., decrease of smoke point, formation of extra volatile and flavor compounds, decrease of interfacial tension). From a nutritional point of view, the compounds formed are not relevant because they are similar to those originated by the action of pancreatic lipase before intestinal absorption. Hydrolysis is the only reaction breaking down the TG molecule, with formation of diglycerides and fatty acids. Although hydrolysis is one of the simplest reactions during frying, inconsistent results on the variables promoting the formation of free fatty acids have been obtained. For some authors, hydrolysis is the most important reaction during frying (Barbanti, Pizzirani, and Dalla Rosa, 1994; Pokorny, 1998). Nevertheless, in well-controlled frying operations of potatoes

under many different conditions, hydrolytic products were minor compounds within the pool of degradation compounds, even though the substrate had a very high content of water (Arroyo et al. 1995; Dobarganes, Marquez-Ruiz, and Perez-Camino, 1993; Perez-Camino et al., 1991; Sebedio et al., 1990). However, used frying oils with high contents of diglycerides and fatty acids from the fast-food sector have also been reported (Masson et al., 1997). These results indicate that other unknown variables have a much more important effect than the moisture content of the food. Frying also affects minor components of the oil. For example, phytosterols degrade into a complex mixture of phytosterol oxides that are difficult to separate and quantify. Thus, phytosterol degradation products are another example of the analytical difficulties involved in the separation and quantification of new compounds formed in frying (Dutta et al., 2006). Also, tocopherols, the major natural antioxidants in refined oils, have been studied due to their rapid loss at frying temperatures (Barrera-Arellano et al., 2002). The most important degradation products of tocopherols have been identified in recent investigations (Verleyen et al., 2001; Verleyen et al., 2002).

2.4.7 VOLATILE COMPOUNDS

During frying, the oil or fat is subjected to high temperatures in the presence of air and water, which results in the formation of a high number of new compounds through thermal, oxidative and hydrolytic reactions. The occurrence of hydroperoxides, the primary oxidation compounds, is very limited due to their great instability at high temperatures. From them, a wide range of secondary oxidation products are formed, as saturated and unsaturated aldehydes, ketones, hydrocarbons, lactones, alcohols, acids and esters. Sulfur compounds and pyrazine derivatives may develop in the food itself or from the interactions between the food and oil.

An important route for formation of new compounds is the hydroperoxide breakdown, which gives rise to volatiles and short-chain compounds attached to the glyceridic backbone that are part of the non-volatile molecules. Whereas the volatiles are largely removed from the oil during frying and

have implications in the flavor of both the frying oil and the fried food, the non-volatile compounds remain in the frying oil and are absorbed by the food modifying the oil nutritional and physiological properties.

The oil flavor developed during deep-fat frying is described as fruity, grassy, buttery, burnt, nutty, and fishy. It depends on the oil and number of frying cycles, but the frying temperature is not significant on the oil flavor (Prevot et al., 1988). The oxidation of linolenic acid during deep-fat frying increases fishy odor and decreases fruity and nutty odor. In general, sensory quality decreases with the number of frying cycles.

Different oils produce different flavor during deep-fat frying, due to the qualitative and quantitative fatty acids differences in the frying oils; for instance, the flavor quality of frying potatoes obtained with peanut oil at 160°C was better than those provided by soybean oil or rapeseed oil subjected to frying at 180°C and 200°C, respectively (Prevot et al., 1988). Wu and Chen (1992) reported that 2-heptenal, 2-octenal, 1-octen-3-ol, 2,4-heptadienal, and 2,4-decadienal were the major volatile compounds in soybean oil at 200°C.

Typical desirable fried flavor is produced at the optimal concentration of oxygen. Low amounts of oxygen produce poor and weak flavor, and high levels of oxygen produce off-flavors (Pokorny, 1989).

Due to the limited unsaturation degree of the frying oils and fats, oleic and linoleic are the two main unsaturated fatty acids undergoing degradation and the expected volatile compounds are those coming from their major hydroperoxides.

Linoleic acid is mainly responsible of fried flavor compounds in fried foods, like dienal, alkenals, lactones, hydrocarbons, and various cyclic compounds (Pokorny, 1989).

The oxidation of linoleic or linolenic acids lead to the production of desirable flavor compounds found in frying oil (Buttery, 1989), such as 4-hydroxy-2-nonenoic acid and its lactone, 4-hydroxy-3-nonenoic acid and its lactone, *trans,trans*-2,4-decadienal, *trans,trans*-2,4-nonadienal, *trans,trans*-2,4-octadienal, *trans*-2-heptenal, *trans*-2-octenal, *trans*-7-octenal, nonenlactone, and trienals.

On the other hand butanal, pentanal, hexanal, heptane, pentanol, 2-hexenal, heptanal, 1-octen-5-ol, 2-pentylfuran, and 2-decenal provide off-odors in deep-fat frying (Prevot et al., 1988).

Petersen et al. (1999) identified pentanal, hexanal, nonanal, 2-octenal, 2,4-heptadienal, 2-nonenal, 2,4 nonadienal and 2,4 decadienal as compounds contributing to boiled potato off-flavors. Recently, Blanda et al. (2010) found a strong correlation between boiled potato off-flavors and a high content of 2-pentenal, 2-hexenal, 2 heptenal, 2-pentylfuran and 2-decenal.

In addition to the frying oil, other source of volatiles include oxidative and thermal decomposition of the lipids in the food itself: carbonyl compounds formed during deep-fat frying can react with amino acids, amines, and proteins and produce desirable and nutty pyrazines (Negroni et al., 2001), and sulfur compounds may develop from interaction among these products with phospholipids.

Some volatile compounds formed during deep-fat frying (such as 1,4-dioxane, benzene, toluene, and hexylbenzene), do not contribute to desirable flavor and are actually toxic compounds (Koschutnig, 2007).

There are many reports on the compounds responsible for the flavor of fried foods. For instance, it appears that the desirable flavor of fried potatoes arises from the alkyl pyrazines, alkyl thiophenes and alkyltyazoles produced through the reactions of amino acids, sugars and lipids and their breakdown products. The qualitative composition of volatiles formed by lipid autoxidation is described in a limited number of studies where oils and fats are thermoxidized in the absence of foods. A complex mixture of compounds of different structure and polarity (including hydrocarbons, alcohols, ketones, lactones, aldehydes, acids, etc) is obtained (Gillat, 2001), among which those included in Table 1 are the major compounds (Dobarganes, et al., 1986) that give rise to the other compounds in a secondary step.

Table 1 Volatile compounds formed by decomposition of hydroperoxides through homolytic scission of the alkoxy radical on either side of the carbon bearing the oxygen (A or B). Adapted from www.lipid.library.aocs.org

Fatty acyl group	**Hydroperoxides**	**VOLATILE COMPOUNDS**		
		Route A*	Route B**	
			+ OH·	+ H·
Oleate	8-ROOH	undec-2-enal	decanal	dec-1-ene
	9-ROOH	dec-2-enal	nonanal	non-1-ene
	10-ROOH	nonanal	octanol	octane
	11-ROOH	octanal	heptanol	heptane
Linoleate	9-ROOH	deca-2,4-dienal	non-3-enal	nona-1,3-diene
	13-ROOH	hexanal	pentanol	pentane

* Scission on the bond closer to the carboxylate function.
** Scission on the bond closer to the methyl group. Radicals produced further react with radicals OH or H .

As mentioned before, volatiles are removed at the high temperatures of the frying process and the composition of volatiles depends on both fatty acid composition and the level of degradation of the oil. Unfortunately, due to the nature of the volatile fraction and to the different concentration techniques used, quantitative gas chromatography results are difficult to obtain and they are normally expressed as percentages of the total chromatographic area. The six aldehydes obtained by route A (hexanal, 2,4-decadienal, octanal, nonanal, 2-decenal and 2-undecenal) are always present in the volatile fraction, suggesting a significant oxidation of oleic and linoleic acids even in oils that are rich in linoleic acid. Among them, 2,4-decadienal is considered the major contributor to deep-fried flavor. As expected, high levels of octanal, nonanal, 2-decenal and 2-undecenal are found in oils rich in oleic acid (i.e. olive, palm oil and high-oleic sunflower oil).

Finally, all the compounds obtained are those also present in oils and fats oxidized at room temperature during storage and that contribute to rancid odor.

Unfortunately however, work remains to be done to establish the relationships between volatile composition with sensory attributes, pleasant or undesirable perception, highly related to odor threshold values, and concentration and interactions of the different compounds in the complex mixture of volatiles.

2.5 FRYING OILS

The fatty acid composition of dietary fat is of great importance from the technological point of view: in fact it is fundamental to the preservation of the product. The ease with which a fat undergoes a process of oxidative rancidity depends largely on the degree of unsaturation and therefore it remains conditional on the behavior of the fat that undergoes several steps for the preparation of food, such as cooking and frying, because unsaturated oils have a very significant risk of curing treatments at high temperatures. The quality of fried foods depends not only on the type of foods and frying conditions, but also on the oil used for frying. Thus, the selection of stable frying oils of good quality is of great importance to maintain a low deterioration during frying and consequently a high quality of the fried foods.

Many refined oils and fats are used for frying and the ideal oil composition may be different depending on technical or nutritional considerations. In general, the decision is influenced by many factors amongst which functionality, nutritional properties, cost and availability stand out. Palm olein and partially hydrogenated oils have been considered the most stable oils for frying although, in the last decades, development of genetically modified seeds containing oils with a lower degree of unsaturation than those of the traditional oils has significantly increased the availability of oils of high thermostability in the marketplace (Hazebroek et al., 2000; Marmesat et al., 2008).

2.5.1 PALM OIL

The plant is native to Africa but has spread to Asia, America and Europe, it looks like a date palm with a large top of fronds that grow from reading a sturdy trunk. The fruit grows in clusters, each of which contains from 200 to 2000 individual fruits, the mesocarp which is obtained by centrifugation is the crude oil to the hydraulic pressure of the pulp is treated appropriately, with a yield of 75%

compared to the dry weight pulp; within this shell contains a two or three stones, from which we obtain a different type of oil ("palm kernel oil"). The crude oil is orange due to carotenoids (500-700 ppm, 90% of which consist of α - β-carotene) (O'Brien, 1998), and of different texture depending on the type of extraction used, it is then neutralized, bleached and deodorized in a single stage, is also often subjected to partial hydrogenation.

This oil is characterized by a high content of saturated fatty acids and is solid at room temperature is not very common in Mediterranean-type diet.

Among the constituents glyceridic is definitely mentioned as vitamin E, a fat-soluble vitamin, whose main components are two homologous series, known as tocopherols and tocotrienols, the vitamin E content in palm oil crude hovers between 600 and 1000 ppm, of which the tocopherols and tocotrienols represent the 18-22% 78-82% (α - δ - γ-tocotrienol). Compared with oils rich in polyunsaturated fatty acids, palm oil is more resistant to oxidation due to its higher content of saturated fatty acids, as can be seen from the tables of composition, in addition, it contains natural antioxidants such as tocopherols, tocotrienols and carotenoids: for they are able to inactivate the factors that promote oxidation or to interfere in one or more steps of the reaction, and their actions are either to "wipe" the free radical-preserving biological membranes and cellular-aging suppress the singlet oxygen. In particular, carotenoid singlet oxygen quenching by energy transfer, while the extinction mechanism of tocopherols is via charge transfer (Sambanthamurthi et al., 2000). But the tocotrienols are not only antioxidants: fact, recent research has shown that, besides protecting cellular aging, they also have potentially therapeutic effects: first, tocotrienols have hypocholesterolemic effects, anti-thrombotic and anti-tumor, and this clearly indicates that can be used in the prevention and / or degeneration in the treatment of cardio-vascular (Theriault, 2000), was also seen that they are also inhibitors of mammary carcinogenesis (Guthrie, 2000).

2.5.2 HIGH OLEIC SUNFLOWER OIL

From a variant of normal sunflower *"Helianthus annuus L."*, not containing GMOs, have been produced in recent years of sunflower seeds are high in oleic acid, whose oil shows a fatty acid composition similar to that of' olive oil, with all the benefits of high content of monounsaturated fatty acids (MUFA), such as oleic acid. The traditional sunflower oil contains 68% linoleic acid (C18: 2) and about 20% oleic acid (C18: 1), is defined as high-oleic sunflower oil it contains about 80% oleic acid. Under the nutritional recommendations, was adopted the high oleic sunflower oil, preferring high levels of MUFA and low levels of saturated fatty acids (SFA), hydrogenated oil as an alternative stable. The Mediterranean diet is known for its protective effects by helping to prevent a myocardial infarction. This fact nutritional model recommended the consumption of foods rich in MUFA rather than SFA. The high-oleic sunflower oil, in this case, the resource is cheaper than the MUFA diet aimed at preventing heart disease. The replacement of dietary saturated fatty acids with MUFA significantly reduced the concentration of LDL (the so-called bad cholesterol). The high-oleic sunflower oil has a neutral taste and high oleic acid content gives an excellent oxidation stability without hydrogenation, resistance to high temperatures and lack of unpleasant odors during frying. For these reasons, the high oleic sunflower oil is widely used for this purpose.

2.5.3 EXTRAVIRGIN OLIVE OIL

The extravirgin olive oil is a major source of fat in the Mediterranean diet and has a very peculiar chemical composition and nutritional quality completely than other oils obtained from seeds. This oil is obtained from olive drupes of Olea Europea The plant, belonging from the Oleacea family. The cultivation of olive trees dates back to biblical times and today along with its oil production, remains in the Mediterranean basin, where is essential for the agricultural practice. Unlike other vegetable oils, the oil is obtained only through mechanical phases (crushing, mixing and separation

of the phases obtained) is then consumed without any refining or other chemical treatments. The fatty acids contained in olive oil have an intermediate degree of unsaturation; also low in saturated fatty acids is advantageous from the point of view nutritional and health.

The extra virgin olive oil is chemically made up of 98% from saponifiable fraction, which represents almost all of triglycerides, esters of glycerol with fatty acids, whose composition is represented by monounsaturated fatty acids in an average amount equal to 75%, (with prevalence of oleic acid) from saturated fatty acids in the average amount equal to 16% (of which there is a predominance of palmitic acid 7-15% and 2-6% fraction of stearic acid) polyunsaturated fatty acids an average amount equal to 9% (with a predominance of linoleic acid and alpha-linolenic limited quantities). The remaining 1-2% are unsaponifiable fraction which is constituted from phenols, polyphenols, tocopherols, and triterpenic alcohols, aliphatic, chlorophyll, vitamins A, D, E, K.

2.5.3.1 NUTRACEUTICAL ASPECTS OF OLIVE OIL

The special flavor to the food given extra virgin olive oil and its components, makes the most delicious dishes, pleasant and attractive. This contributes to secretory stimuli activate the digestive system by promoting a better digestibility and metabolism of a good tolerance gastric and intestinal. It is also well known that extra virgin olive oil, for its high content of monounsaturated fatty acids, particularly oleic acid, protects the gastric mucosa, decreases the secretion of hydrochloric acid (important for gastric or duodenal ulcer) inhibits secretion of bile, improves emptying of the gallbladder bile preventing the formation of stones, produces less pancreatic secretory activity (important in diseases such as pancreatitis) facilitates the absorption of fat soluble vitamins and calcium, exerts a laxative (in particular fasting) and helps to correct chronic constipation. For the action associated with the minor constituents, reduces the risk of certain autoimmune diseases and breast cancer and colon cancer. The oleic acid in the diets of extra virgin olive oil, interferes positively on the processes of biosynthesis and metabolism of cholesterol. Maintains low or reduced

levels of both total cholesterol (10% reduction), is bound to LDL cholesterol, the so-called bad cholesterol (14% reduction) and triglycerides (13% reduction) and reduce blood pressure. But does not decrease levels of cholesterol bound to HDL, the so-called good cholesterol, a garbage collector, which avoids the accumulation of fat in the walls of arteries. Administration of extra virgin olive oil from place: the replacement of dietary saturated fatty acids with monounsaturated to adequate intake of polyunsaturated fatty acids, the reduction in the proportion of lipids that undergoes oxidative processes, optimal contributions to minor compounds, the reduction of LDL in plasma and arterial walls. These results are not obtained but diets containing sunflower oil rich in oleic acid also made. This shows that oleic acid alone is not sufficient and it is essential to the association and interaction with other factors present in extra virgin olive oil. The fatty substances containing excessive amounts of saturated fatty acids, mainly palmitic acid (found in animal fat and palm fat 41-48%) and stearic acid, contained mainly in solid fats such as butter (60-78% of saturated fat), lard (20-60% saturated fatty acids), margarine solid (33.8 to 71.5% saturated fatty acids) and tallow. Saturated fats if taken in quantities exceeding those usually proposed, promote early childhood, weight gain, obesity up, raising the rate of LDL cholesterol and blood, facilitating the changes of the arteries, heart disease , some cancers and various inflammatory diseases. Polyunsaturated fatty acids, linoleic acid, linolenic acid, are also considered as functional foods as a precursor of many cytokines acting stimulant and vasoconstrictor vessel, pro-and anti-inflammatory, inhibiting or stimulating the immune response as needed. Are contained in extra virgin olive oil a ratio similar to that of breast milk and therefore very likely to be optimal proportions for the needs of the organism from the earliest stages of life. Are adequately protected by vitamin E (alpha-tocopherol) in combination with polyphenols and other antioxidants which play saving action of the molecules of alpha tocopherol. It is quite evident then the importance of their presence in the Nutraceutical foods but in optimum ratios to maintain a good health condition. From what has just been exposed, and many other investigations, it is clear the importance of balance in extra virgin olive oil between the two essential fatty acids and their long chain derivatives (products

for the composition of the structure of cells and their membranes), for the functionality of the brain, for the development of neuro-psycho-motor acquisitions, the structuring of the retina, for the production of many proinflammatory cytokines and anti-inflammatory etc.. Therefore, the introduction in the diet of olive oil extra virgin oil, weaning and until the child begins to introduce the fish, is the only source that supplies the rapidly evolving body a certain amount of omega 3. The particular composition of extra virgin olive oil, and the presence of adequate amounts of antioxidants, is particularly useful for the health of the organism in the preparation of foods that must undergo the cooking and / or frying. During cooking, in fact, all the lipids in the presence of atmospheric oxygen undergo an acceleration of the phenomenon of oxidation with the formation of significant amounts of free radicals that have toxic effects. The phenomenon, which is delayed by the presence of antioxidants, while the more pronounced the higher the degree of unsaturation of fatty acids and the length of cooking time. Moreover, each has its own fat point tolerance to heat, smoke or point called critical temperature, beyond which the glycerol content in triglycerides, decomposes into acrolein. This is a very harmful substance to the liver and in particular, when the firing lasted for a long time, also form other toxic substances. Since olive oil, rich in the monounsaturated oleic acid, as refined as any other oil has a smoke point of about 210 ° C, higher than the most widely used vegetable oils (coconut and palm oil), the margarine and butter, it follows that it is the best for cooking, especially for frying. Even the production of secondary oxidation products (aldehydes and ketones) are lower when compared to other cooking oils. The seed oils, as rich in polyunsaturated fatty acids, particularly omega-6 linoleic acid, the presence of double bonds are highly unstable and do not bear the combined attack of oxygen of high temperatures. The antioxidant potential decreases with increased heating in soybean oil and sunflower oil compared with extra virgin olive oil. The extra virgin olive oil plays Healthy action even when it is used for bakery products and confectionery in the place of "vegetable oil" or "margarine from vegetable oils." In fact, the vegetable oils used for such products are usually palm oil and coconut, on the contrary, what you think it is of plant origin, are made up primarily of

saturated fatty acids (on average more than 85% saturated fat for coconut oil) and the body can use it without any major problems up to a maximum of 20 grams per day. This dose is easily accessible if you think that with 100 g of potato chips and snacks from the market if it can take up to about 19 g and others are also taken during the day with milk, cheeses, meats and condiments including the butter. Margarine of vegetable oils (from 33.8 to 71.5% saturated fatty acids) is produced by the food industry to use unsaturated oils to produce solid fats and low-cost very interesting for the baking industry. Beyond that hydrogenation helps to prevent them from going rancid. During production, the process of hydrogenation artificially breaks one of the two unsaturated double bonds, adds hydrogen and saturated lipids from place to hydrogenated. Together with the saturation of double bonds, you always get a certain amount of isomerized fatty acids: trans fatty acids. These saturated fatty acids, are used by the body to protect cell membranes as if they were good, but fail, so that the membrane no longer functions properly in the management of minerals and nutrients that pass through it with a functional deficit of cells. Even the butter is rich in saturated fatty acids (60-78% of saturated acids) for which, particularly in the first year of life when the child eats a lot of milk rich in these lipids, it is not used as a food to which it would increase the contribution. After the year can be added from time to time in the childs' diet of cakes made with butter, preferably homemade.

2.6 ENCAPSULATION PRODUCTS

In recent years, much emphasis has been placed on the production of functional food, nutraceutical compounds and enriched food, with the aim to improve and compensate the nutrition shortages resulting from lifestyle or generated by cooking methods and/or technological processes. In order to ensure their integrity and release, the compounds with antioxidant activity can be encapsulated in several systems that can warrant their protection from the environment, as well as their bioavailability.

The utilization of encapsulated polyphenols instead of free compounds can overcome their unstability drawbacks, alleviate unpleasant tastes or flavors, and improve their bioavailability and *in vivo* and *in vitro* half-life (Fang et al., 2010). Bioactive lipids like antioxidants can be incorporated into aqueous based food, but often several technical challenges have to be overcome first, depending on the different molecular form of the lipophilic bioactive compound (Mc Clements, 2010). Consequently, different types of delivery systems have been developed, like simple solutions, association colloids, emulsions, biopolymer matrices and powders, which all have their advantages and disadvantages (Mc Clements et al., 2009).

Encapsulation of food is a practice known and applied in the food industry for over 60 years. It is a technology that allows you to enclose materials in nature in solid, liquid or gas in controlled-release capsules in specific conditions. Microencapsulation is defined as a technology of packaging solids, liquids, or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions. Nanotechnology in recent years has developed into a wide-ranging, multibillion-dollar global industry. The global market impact of nanotechnology is widely expected to reach 1 trillion US$ by 2015, with around 2 million workers (Roco and Bainbridge, 2001)

2.6.1 WHY ENCAPSULATION?

The food industry is ultimately driven by profitability, which is consequent on gaining consumer acceptance by offering added-value in terms of quality, freshness, new tastes, flavours, textures, safety or reduced cost. The purpose of encapsulation is to protect its contents from the environment which can be destructive while allowing small molecules to pass in and out of the membrane (Gibbs et al., 1999). For many years, this technique has been used in the pharmaceutical industry for time-release, enhanced stability of formulations and flavor masking. Prescription drugs, over-the-counter drugs, vitamins and minerals have been encapsulated. Therefore, these applications, in addition to many others, would be useful in the food industry (Gibbs et al., 1999)

The food industry applies encapsulation process for a variety of reasons: (1) encapsulation=entrapment can protect the core material from degradation by reducing its reactivity to its outside environment (e.g.,heat, moisture, air, and light), (2) evaporation or transfer rate of the core material to the outside environment is decreased=retarded, (3) the physical characteristics of the original material can be modified and made easier to handle, (4) the product can be tailor to either release slowly over time or at a certain point (i.e., to control the release of the core material to achieve the property delay until the right stimulus), (5) the flavor of the core material can be masked, (6) the core material can be diluted when only very small amounts are required, yet still achieve a uniform dispersion in the host material, and (7) it can be employed to separate components within a mixture that would otherwise react with one another (Kasappa et al., 2005).

Many of the current nanotechnology applications in the food sector appear to have emerged from related sectors, such as pharmaceutical, cosmetics and nutraceuticals (Chaundry., et al., 2008).

Various techniques are used for encapsulation (Dziezak, 1988). In general, three steps are involved: formation of the wall around the material, ensuring that leakage does not occur, and ensuring that undesired materials are kept out. These encapsulation techniques include spray drying, spray

chilling or spray cooling, extrusion coating, fluidized bed coating, liposome entrapment, coacervation, inclusion complexation, centrifugal extrusion and rotational suspension separation.

2.6.2 LIPOSOME ENTRAPMENT

Molecular vesicles for antioxidant substances could be liposomes, spherical bilayers. They are built up if polar lipids, whose source is usually soy- or egg- lecithin, are dispersed in an aqueous medium. Lecithin is a common emulsifier and texture modifier in food and is generally recognized as safe (Laye et al., 2008). Liposomes are suitable carrier systems for both, water- and oil-soluble components like antimicrobials, flavors, antioxidants and bioactive ingredients, because they are biocompatible, biodegradable, nontoxic and able to release the encapsulated substance when it is demanded (Benech et al., 2002; Gòmez-Hens et al., 2006). They help overcoming barriers, directing contents towards specific sites *in vivo* (Mady et al., 2009). It has been demonstrated that an encapsulation with liposomes promotes the biological activity of antioxidants probably by alleviate intracellular uptake and extending their half-lives (Mozafari et al., 2006).

They consist of one or more layers of lipids (Figure 2.5) and thus are nontoxic and acceptable for foods. Permeability, stability, surface activity and affinity can be varied through size and lipid composition variations. They can range from 25 nm to several microns in diameter, are easy to make and can be stored by freeze-drying (Gibbs et al., 1999).

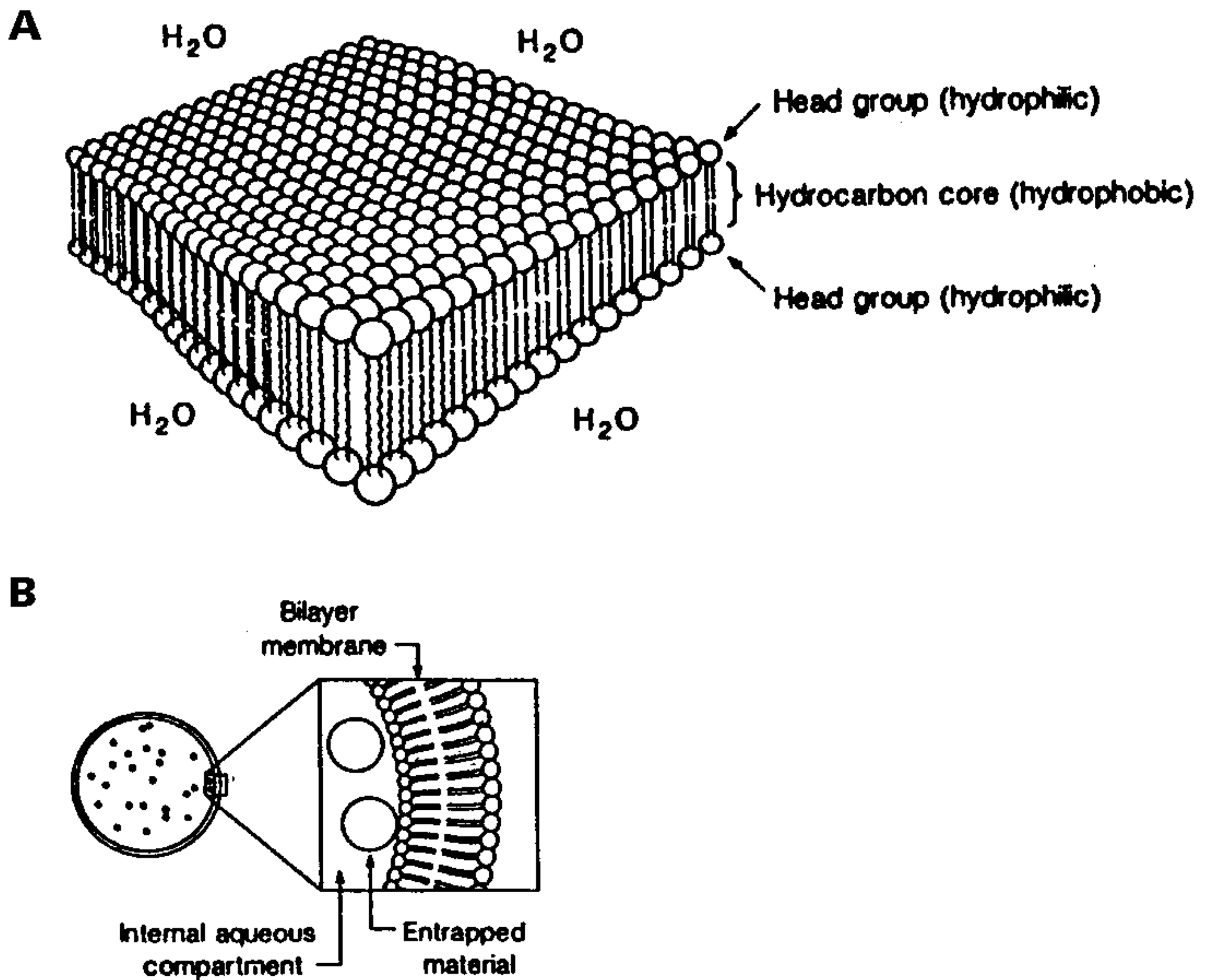

Figure 2.5 Schematic diagram of a sheet of lipid bilayer (A) and the liposome formed from the lipids (B) (adapted from Reineccius, 1995).

Liposomes or lipid vesicles are aggregates formed from aqueous dispersions of amphiphilic molecules such as polar lipids that tend to produce bilayer-structures (Lasch et al., 2003). Liposomes that contain only a single bilayer membrane are called small (<30 nm) or large (30– 100 nm) unilamellar vesicles, or SUVs and LUVs, respectively (New, 1990b). Liposomes that contain more than a single bilayer membrane are referred to as multilamellar vesicles (MLV) if all layers are concentric, or multivesicular vesicles (MW) in which case a number of randomly sized vesicles may be enclosed in the interior of another vesicle (Figure 2.6) (New, 1990b). Liposomes larger than

~300 nm will scatter light sufficiently to allow them to be seen by the naked eye and these samples will have a cloudy white appearance.

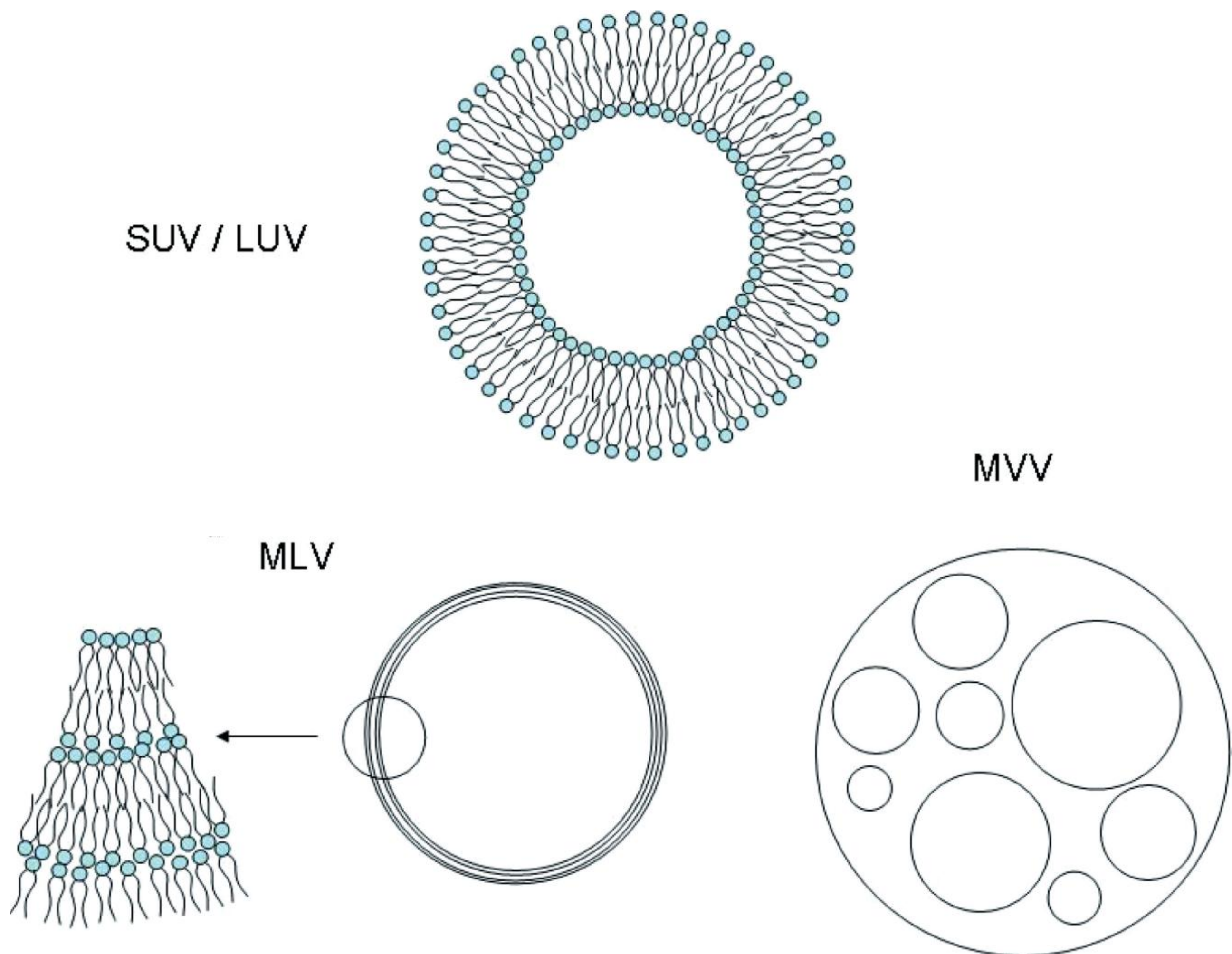

Figure 2.6 Schematic representation of the structure of large and small unilamellar vesicles (SUV/LUV), multilamellar vesicles (MLV) and multivesicular vesicle (MVV).

Chemically, liposomes are primarily composed of phospholipids, although other lipids such as galactolipids may also be incorporated. The major components of biological membranes, phospholipids exist as either sphingolipids or phosphodiglycerides (Barenholz et al., 1977; Lasch et al., 2003).

2.7 HETEROCYCLIC AROMATIC AMINE

2.7.1 FORMATION

In recent years, more emphasis was placed on the use of healthy eating, in fact, the nutrition plays a vital role in maintaining a good state of health. There have been recent numerous studies that highlight the role of cause and effect between diet and disease. There are several harmful substances in food and a lot of importance among these is covered by heterocyclic aromatic amines (HAA). That's are the most potent mutagenic substances ever tested in the Ames/*Salmonella* mutagenicity test (Felton et al., 1990). The coocking process is responsible for the formation of HAA from the macromolecular component of food and determinant is the cooking time and the temperatures. HAA are frequently formed in muscle meats during frying, broiling and grilling. The methods that used low temperatures such as stewing, boiling and backing usually don't form HAA (Felton et al., 2003).

Several of them, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline (MeIQx), 2-amino-1-methyl-6–phenylimidazo-[4,5-b]pyridine (PhIP), 2-amino-9H-dipyrido[2,3-b]indole (AαC), 2-amino-3-methyl-9H-dipyrido[2,3-b]indole (MeAαC), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole(Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), and 2-amino-dipyrido[1,2-a:39,29-d]imidazole (Glu-P-2) have been classified by the *International Agency for Research on Cancer* as possible, and one of them, 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ) as a probable human carcinogen (IARC, 1993).

Most of them have an exocyclic amino group, except b-carbolines, harman, and norharman (Jagerstad et al., 1998). In the β-carbolines, it is the lack of such functional group that causes them to be non-mutagenic in the Ames/*Salmonella* mutagenicity test (Sugimura et al., 1982).

These compounds can be divided in two classes IQ-Type (HAA) and non-IQ-type (HAA) based on the polarity and that are formed when creatine, creatinine, sugar and aminoacids are found to be

heated (Sugimura et al., 2000) and their formation appears to be dependent to time, to frying temperature and to type of food cooked.

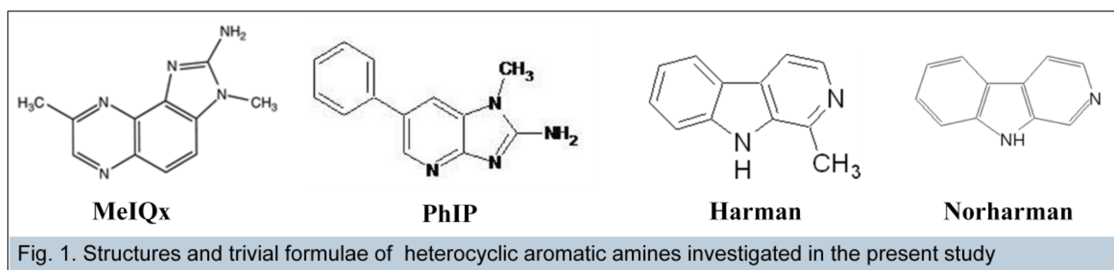

Fig. 1. Structures and trivial formulae of heterocyclic aromatic amines investigated in the present study

IQ- and IQx-types HAs and their derivatives (methylated forms) are suggested to be formed from creatin(in)e, sugars, free amino acids, and some dipeptides through Maillard reaction and Strecker degradation upon heating (Felton et al., 2000; Jagerstad et al., 1983) (Fig. 2.7).Weisburger (Weisburger et al., 1994) found that addition that creatine formed the amino-imidazo part of IQ and IQx by cyclization and water elimination, while the Strecker degradation products such as pyridines or pyrazines formed in the Maillard reaction between amino acids and hexose contributed to the remaining part of the molecule, probably via aldol condensation (Jagerstad et al., 1991) (Fig. 2.7). This reaction is especially favored at temperatures above 100°C (Abdulkarim et al., 1998; Cheng et al., 2006).

Free radical mediated pathway has also been proposed. Hayashi and Namiki showed that, prior to Amadori rearrangement, free radicals may be formed via fragmentation reactions. Further studies suggested the involvement of pyridine and pyrazine radicals (Cheng et al., 2006).

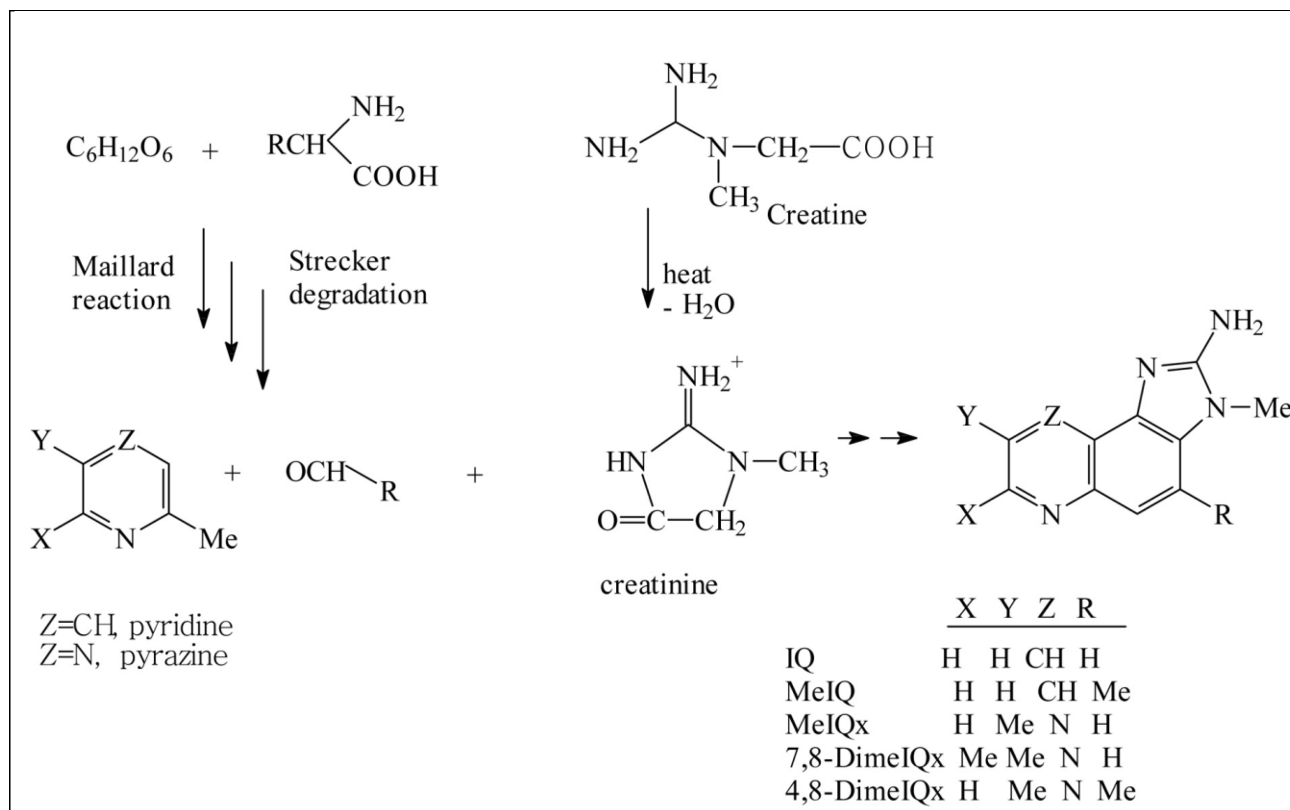

Figure 2.7 Suggested pathway for the formation of imidazo-quinolines and imidazo-quinoxalines (Cheng et al., 2006)

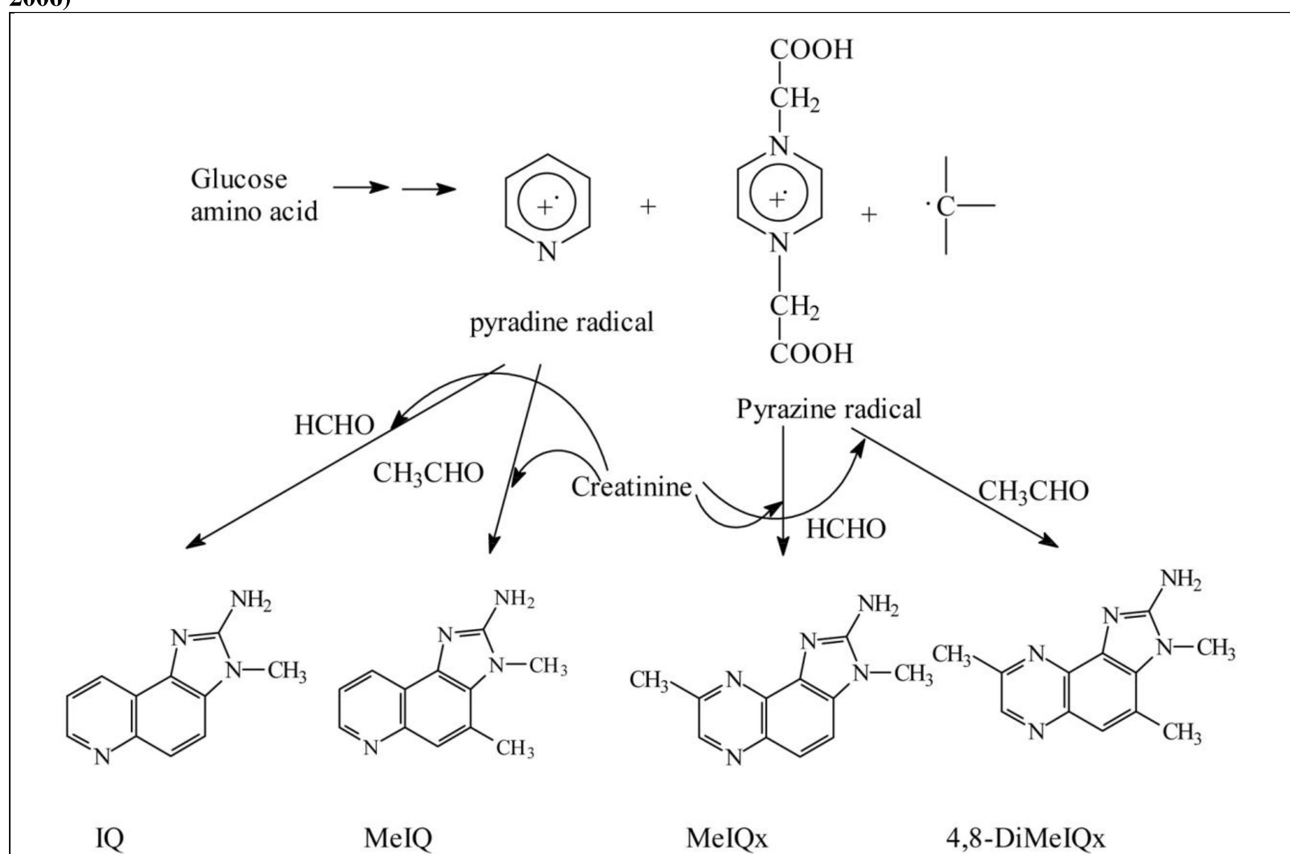

Figure 2.8 Suggested pathway for the formation of imidazo-quinolines and imidazo-quinoxalines (Cheng et al., 2006)

2.7.2 HETEROCYCLIC AROMATIC AMINE AND HUMAN HEALTH

Heterocyclic amines (HAs) are considered the main food mutagens in cooked meat products. Heterocyclic amines (HAs) are a highly mutagenic class of compounds that have been investigated since the detection of mutagenic activity to *Salmonella typhimurium* TA98 in the smoke produced by broiling fish (Felton et al., 2000).

The metabolic activation of heterocyclic amines is catalyzed by N-acetyltransferases (NAT) 1 or 2 (Turesky et al., 2004; Hein et al., 2000), which are coded by genes (NAT1 and NAT2) that are highly polymorphic. Heterocyclic amines can be metabolized more or less efficiently by individuals depending on their NAT genotypes. Based on the underlying mechanisms, it is hypothesized that ''rapid'' NAT1 and/or NAT2 acetylators more readily activate heterocyclic amines to their ultimate carcinogenic forms, thereby amplifying the association of cooked meat and smoking with risk for colorectal cancer. We sought to investigate this hypothesis in a case-control study nested within the Hawaii–Los Angeles Multiethnic Cohort Study (Nothlings et al., 2009). Epidemiological evidence suggests that consumption of well-done or grilled meat may be associated with increased cancer risk in humans. However, the presence of an individual HCA in cooked meat is highly correlated with the presence of other HCAs and with many other constituents, including protein, animal fat, nitrosamines, and substances other than HCAs formed during cooking, such as polycyclic aromatic hydrocarbons. Furthermore, the carcinogenic effects of these HCAs may be inhibited or enhanced by many factors, including interactions of HCA mixtures. It is therefore difficult for human epidemiological studies to establish associations between cancer risk and specific HCAs. For each of these four selected HCAs, the data from epidemiological studies are insufficient to evaluate whether the increased cancer risk is due specifically to consumption of that particular HCA in food (NTP 2002). In rats, orally administered MeIQx also increased the combined incidence of benign and malignant Zymbal-gland tumors (sebaceous-gland adenoma and squamous-cell papilloma and carcinoma) in both sexes, and it caused skin cancer in males and cancer of the clitoral gland in fe-

males (squamous-cell carcinoma in both cases). Case-control studies (one study for each tissue site) suggested that MeIQx may increase the risk of benign colon tumors (adenoma) (Sinha et al., 2001) and lung cancer (Sinha et al. 2000b). MeIQx intake was not associated with cancer risk in case-control studies of urinary-bladder, kidney, or colon cancer (Augustsson et al., 1999).

PhIP caused tumors in two rodent species, at several different tissue sites, and by two different routes of exposure. Oral exposure to PhIP caused lymphoma in mice of both sexes and in male rats. In rats, it also caused prostate cancer (carcinoma) and cancer of the small intestine and colon (adenocarcinoma) and in males and mammary-gland cancer (adenocarcinoma) in females. Case-control studies suggest that PhIP may increase the risk of breast or stomach cancer. However, the association with stomach cancer was based on only one study (De Stefani et al. 1998), and the association with breast cancer was found in two of three studies (De Stefani et al. 1997, Delfino et al. 2000, Sinha et al. 2000a).

2.7.3 GRAPE SEED EXTRACT

Bioactive lipids like antioxidants can be incorporated into aqueous based food, but often several technical challenges have to be overcome first, depending on the different molecular form of the lipophilic bioactive compound (mc Clements, 2010). Consequently, different types of delivery systems have been developed, like simple solutions, association colloids, emulsions, biopolymer matrices and powders, which all have their advantages and disadvantages (Mc Clements et al., 2009). Grape seed extract containing polyphenolic substances is considered to have an anti-oxidative potential, hence it can be used as a nutritive supplement (Chedea et al., 2010). The composition of the ingredients depends on the kind of grape, preparation conditions and climatic circumstances.

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AIM AND DESCRIPTION OF PhD PROJECT

The study conducted during the PhD research was focused on the assessment of various thermoxidative degradations in frying oils. To achieve this objective, it was considered appropriate to firs proceed with a screening of the major frying oils present in the Italian market, followed by the development of two frying blends (made of vegetable oils), which were subjected to two different experimental frying plans at lab-scale, under controlled and standardized conditions. The same frying blends were also utilized under two different catering frying situations (cafeteria and restaurant). Each frying blend was compared with two reference frying oils (palm olein and extra virgin olive oil).

To evaluate the frying performance of each frying oil and blend, different analytical determinations were carried out (fatty acid composition, total tocopherol content and composition, parameters of hydrolytic and oxidative stability, smoke point, polar compounds, composition of volatile compounds); such analysis were performed before, during and at the end of the frying process. The study led to the identification of one of the mixtures here developed as a valid alternative to palm oil, which is widely used for food frying at catering level; in addition, this research allowed a better definition of the modifications (type and entity) that occur during food frying, according to the conditions used. The determination of polar compounds generated during frying were analyzed by both the official method and a high-performance size-exclusion chromatographic method (HPSEC). This part was carried out during a research period at the laboratory coordinated by Dr. MC Dobarganes at the Instituto de Grasa (Sevilla, Spain).

In the last part of the PhD period, the stability and usage of a special marinade, made of grape seed phenolic extracts encapsulated in liposomal systems, was tested on beef patties. The objective was to evaluate the ability of phenol-enriched liposomes to inhibit the formation of heterocyclic aromatic compounds generated by pan-frying. This part of the project was performed during a research period at the laboratory coordinated by Prof. J. Weiss from University of Hohenheim (Germany).

Keywords: deep frying, vegetable oils blend, palm oil, extra virgin olive oil, catering conditions, oxidative stability, hydrolysis, diacylglycerols, total polar compounds.

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Quality parameters of commercial frying oil blends available in the Italian market

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Abstract

The aim of this study was to determine the overall quality of commercial fresh frying oil blends available in the Italian market and destined for domestic use. Oils were characterized by evaluating different composition, hydrolytic and oxidative parameters as well as the smoke point. Frying oils had an oxidative stability index (OSI) time that varied from 7 to 23 h, which was greatly related to their fatty acid (FA) composition. Most samples had a peroxide value (PV) below 2 meq O2/kg of oil and the free acidity value of all frying oils was below 0.5%, as expected for refined oil blends. Total diacylglycerides (DAG) ranged from 1.1 to 4.8%, where the palm oil-containing blends displayed the highest DAG percentage (4.8%) as it is related to its extraction technology. The smoke point varied from 230 to 252°C, according to the FA composition of each frying oil blend. The total polar compounds detected by Testo 265 instrument, ranged from 9% to 17%. In general, all fresh frying blends here evaluated exhibited good and homogeneous hydrolytic and oxidative characteristics; their frying performance, however, will greatly depend on their FA composition, DAG content and initial oxidative status, which will be reflected on their oxidative stability, foam production and polar compounds content.

Keywords: frying oil blends, quality parameters, oxidation, hydrolysis, Italian market

Introduction

Deep frying is a simple and essential food cooking technique, which is very popular due to the typical and peculiar food sensory characteristics (including flavour, texture and appearance) that it produces (Dana et al., 2003); this results in a very high consumers' acceptability, which has led to a large, worldwide fried food production at different levels (food industry, fast food restaurant, catering and domestic use). However, deep-frying is a complex process that involves food immersion in an oil bath kept at high temperatures (between 160°C and 180°C), in contact with air (Gertz, 2000). This type of food processing operation causes a series of physico-chemical modifications in both oil and food, which depend on the nature of the oil, the type of food, the amount of food to be fried and the processing conditions (temperature, time, holding conditions, oil exchange). Hydrolysis, thermoxidation and polymerization are the main reactions that occur during frying, which induce a gradual decrease of the initial oil quality. More than 500 different chemical compounds formed as a result of autoxidation, thermoxidation, pyrolysis and polymerization have been detected in used frying oils (Gertz, 2004). The influence of the degradation products on the physical properties of the frying oil change during the whole frying process (Gertz, 2004). These modifications can generate a series of chemical compounds with adverse nutritional implications and potentially hazardous effects for human health (Marquez-Ruiz & Dobarganes, 1996). It is, therefore, of outmost importance that the vegetable oils or their blends to be used for frying, have a good starting quality, as they will further degrade during the cooking process and will, thus, directly impact the chemical, nutritional and sensory quality of the fried food (Blumenthal, 1991). The evaluation of the quality of crude vegetable oil blends is, thus, a key approach to identify the most suitable strategies to delay their deterioration. For instance, refined vegetable oils with a poor antioxidant content, are more prone to oxidative deteriorations during storage, marketing and, subsequently, during deep-frying. Although there are several vegetable oils that can be used for frying, some of them (such as soybean, sunflower, corn, rapeseed oils) are often judged unsuitable

for continuous frying due to their relative high content of polyunsaturated fatty acids (PUFA) (Gertz et al., 2000). Palm oil is the most widely used vegetable oil for industrial food frying, since it is very stable towards oxidation due to its elevated percentage of saturated fatty acids (SFA)/monounsaturated fatty acids (MUFA). In addition, some mono-seed oils, such as peanut and olive oil, also represent a good frying alternative, since they have a high percentage of MUFA that makes them more stable with respect to oxidation. Besides these single vegetable oils, there are, nowadays, a wide range of frying products available in the market; these products are designed to combine the best features of the single oils and, in some case, to partially overcome some negative aspects. For instance, the palm oil has an elevated melting point (46.5°C) (Jin et al., 2008), which renders it solid at room temperature, thus being impossible to pour it into the fryer; to overcome this aspect, palm oil has been mixed with other vegetable oils having a higher unsaturation degree, to bring down the melting down and to give rise to a more fluid blend that could satisfy the consumers' requirements. Another important aspect is the level of total polar compounds (TPM), which is considered the best indicator of the presence of degradation products in frying oils (Ruiz et al., 1996; Bansal et al., 2010); in fact, if the TPC content of the frying oil is $\geq$ 25%, the oil should be discarded according to the CM 1991 (CM 1991, No.2568/91).

Although the quality and stability of the single edible oils has been widely studied and characterized, to the best of our knowledge, there is no available information about the overall quality of commercial fresh frying oil blends available in the Italian market and destined for domestic use. The aim of this study was to determine the overall quality of commercial frying oil blends available in the Italian market and destined for domestic use. Oils were characterized by evaluating different composition, hydrolytic and oxidative parameters, as well as their smoke point.

Materials and Methods

Samples

All oil blends were purchased in several Italian supermarkets (Bologna, Italy), so as to obtain a more representative picture. Two bottles per each blend were purchased. Table 1 describes the general composition of the oil blends described in the product labels and the code used for their identification.

Reagents, solvents, and standards

Diethyl ether, double distilled water, *n*-hexane, chloroform, isopropanol, methanol, glacial acetic acid, phenolphtalein, sodium thiosulfate and starch water, were supplied by Carlo Erba Reagenti (Milano, Italy). Anhydrous sodium sulfate was purchased from BDH (BDH, England). Commercial standards of diacylglycerols (dimyristin, dipalmitin, distearin, diolein), were supplied by Sigma Aldrich (St. Louis, MO, USA). Silica solid phase-extraction (SPE) cartridges NH_2 (500 mg stationary phase/3 mL) were purchased from Phenomenex (Torrence, CA, USA). The silylation mixture was prepared with dried pyridine (ACROS Organics, USA), hexamethyldisilazane (BDH, England) and trimethylchlorosilane (Carlo Erba Reagenti, Milan, Italy) at a ratio of 5:2:1 by volume.

Determination of total fatty acid composition

Total fatty acid were determined according to the European Commission Allegate X. B. Regoulation No. 2568/91 (EC Commission Regulation EEC No. 2568/91 of July 1991).

About 30 mg of oil were dissolved in 1 mL of *n*-hexane and then transmethylated with 50 mL of 2 N KOH solution in methanol. The mixture was vigorously shaken with a vortex for 1 min, then

added with a known amount of internal standard solution (50 μL of tridecanoic acid methyl ester), centrifuged at 395×*g* for 3 min and injected into a GC (1 μL of the supernatant).

The GC instrument was a Carlo Erba HRGC Fractovap 4160 (Carlo Erba, Milan, Italy), with a split-splittless injector and a flame ionization detector (FID). A fused silica capillary column (30 m x 0.25 mm i.d. x 0.2 μm of film thickness) coated with a 90% biscyanopropyl–10% phenylcyanopropyl-polysiloxane (Restek, Bellefonte, PA, USA), was used. The oven temperature was programmed from 100°C to 240°C at 4°/min; the final temperature was kept for 20 min. The injector and detector temperatures were both set at 250°C. Helium was used as carrier gas at 1.09 mL/min. The split ratio was 1:70. Peak identification was carried out by comparing the peak retention times with those of the GLC 463 FAME standard mixture. The internal standard method was used for the quantification of fatty acids. The GC response factor of each fatty acid was calculated by using the GLC 463 FAME standard mixture and the internal standard (tridecanoic acid methyl ester, C13:0). Two replicates were analyzed per sample.

Determination of free acidity (FA)

Free acidity value (expressed as % oleic acid) was evaluated according to the official method described in annex III of EC Regulation 2568/91 (EC Commission Regulation EEC No. 2568/91 of July 1991). Two replicates were analyzed per sample.

Determination of total diacylglycerol content (DAG)

DAG were determined according to a modified version of method suggested by Bonoli et al. (2007), where dihydrocholesterol was used as internal standard. Seventy μL of a solution of dihydrocholesterol (1.052 mg of dihydrocholesterol in 1 mL of *n*-hexane:isopropanol (4:1, v/v))

were added to 100 mg oil and dissolved in 500 μL of *n*-hexane before loading into SPE. The rest of DAG purification by SPE elution was the same as reported by Bortolomeazzi et al. (1990). The purified fraction was then silylated (Sweeley et al., 1963), dried under nitrogen stream and dissolved in 100 μL of *n*-hexane. One microliter of the silylated solution was injected into a gas chromatograph (GC 8000 Series Fisons Instruments, Milano, Italia) with injector spit-splittless and a flame ionization detector (FID). A fused silica capillary column (25 m x 0.25 mm i.d. x 0.1 μm of film thickness) coated with 65% diphenyl-polysiloxane–35% dimethyl-polysiloxane (TAP, Varian, Lake Forest, USA), was used. The oven temperature was programmed from 160°C to 350°C at 3°/min and kept a 350°C for 20 min. The injector and detector temperatures were set at 350°C. Helium was used as carrier gas at a flow of 1.40 mL/min (80 kPa of pressure); the split ratio was 1:30.

Identification of DAG was carried out by comparing the peak retention times and the GC trace with those of the DAG standards and chromatograms reported in literature.

Oxidative Stability Index (OSI) of the vegetable oil blends

Oxidative stability of samples were determined according to the AOCS Official Method Cd12b.92 (AOCS Official Method Cd 12b-92). The analysis were carried out by Omnion Oxidative Stability Instrument with eight channel (Omnion, IL). A 5.0 ± 0.1 g of oil were placed in a polycarbonate tube and heated at 110±1°C under atmospheric pressure and at 150 mL/min of air flow rate. This test is based on the increase in conductivity due to the formation of volatile acids (mainly formic acid) during oil accelerated oxidation (Jebe et al., 1993). The conductibility was measured in polycarbonate tubes using twice distilled water. Results were expressed as induction time (h). Two replicates were analyzed per sample.

Determination of peroxide value (PV)

Peroxide value (PV) (expressed as meq O_2/kg oil) were evaluated according to the official method described in annex III of EEC Regulation 2568/91 (EC Commission Regulation EEC No. 2568/91 of July 1991). Two replicates were analyzed per sample.

Determination of total polar compounds (TPC)

Total polar compounds were measured by Testo 265 (electronic cooking oil tester) (CP B 04/11/512 PRO 04/11/015). The sensor based on parallel plate capacitor was immersed in the hot oil at frying temperature for about 5 min, after completion of the frying cycles. The TPC and temperature readings were carried out once the sensor had stabilized (around 1 min). The Testo 265 instrument provided a TPC value (expressed as %), with a $\pm$ 2% of standard deviation of the instrumental measurement. Two replicates were analyzed per sample.

Determination of the smoke point

This determination was performed according to the NGD C77 method (1976), by using a Cleveland instrument. The smoke point is directly correlated to the fatty acid composition of the oil; in fact, lower smoke points are generally measured in oils and fats with a high content of PUFA, due to the lower stability of the double bonds at high temperatures. The smoke point, therefore, is an important determination to assess the degradation level of an oil/fat when subjected to heating. Briefly, the oil is placed in an brass container, up to the knurling fixed level. The oil is heated and, when it starts smoking, the temperature is registered. Two replicates were analyzed per sample.

Statistical analysis

The data are reported as mean values of 2 independent replicates (n= 2) of each analytical determination. One-way ANOVA was performed, in order to compare data obtained for different different oils.

Results and Discussion

Fatty acid composition

Table 2 and table 3 reports the main FA present in the frying edible oil blends, as well the three fatty acids classes (according to their unsaturation degree). In SO/HO, PO/SO and HOSO/RO/SO blends, oleic acid was the most abundant FA (78.9%, 63.0% and 60.5%, respectively); in these oil blends, MUFA was the most representative FA class, which is mainly related to the presence of hazelnut, palm olein and high-oleic sunflower oils. In fact, this mixture was from different vegetable oil in different percentage in the blend that reflect the most abundant fatty acid detected for each. SO/SBO/PO and SO/PeO were characterized by a high level of linoleic acid (49.6% and 55.2%, respectively). In this oils blend the PUFA class was the most representative fatty acid class.

In the commercial HOSO/SO blend, unlike the other products here analyzed, both MUFA and PUFA were present at similar levels and were the most representative FA classes; oleic and linoleic acid were the most abundant FA detected (42.1% and 47.5%).

The different FA distributions of all frying blends have to related to the corresponding contribution of the most representative FA of the single oils that constitute the frying oil blends.

Table 3 reports the total unsaturation of the commercial oil blends, which varied from 191 to 533. This index is calculated by multiplying the relative percentage of the various unsaturated FA by different factors (1, 10 and 20 for mono-, di- and tri-unsaturated FA) and it provides an indication of the oil stability as they are inversely correlated. It is interesting to note that the SO/HO, PO/SO and HO/RO/SO blends are characterized by a higher percentage of MUFA classes, only for SO/HO and PO/SO were detected a lower level a total unsaturation (201 and 225, respectively). The total unsaturation of HO/RO/SO was twice as much those of the other blends.

That's parameter have to correlated with the highest percentage of linolenic acid present in this oil mixtures which being the PUFA classes determinant for the total unsaturation.

In fact, the total unsaturation detected was near to that detected for the commercial oils where the PUFA is the FA classes representative. Usually this high amount can be seen as a problem as a resistance an oil's resistance to oxidation, especially when the oil is used at high temperature (Chu & Kung, 1998). In addition, the ratio PUFA/MUFA calculated is specified to be among lowest (0.1 and 0.2, respectively).

Although a high PUFA content is not the best feature for a good oil frying performance, it is important, on the other hand, to remember that PUFA, such as linolenic and linoleic acids, are fundamental in human diet as they cannot be produced by animal metabolism (Tuberoso et al., 2007; Shahidi & Wanasundara, 1998). Essential FA, in fact, carry out structural functions, because they are involved in the regulation mechanism of the cellular structures. They are precursors of eicosanoids, the chemical mediators at the cellular level, and help regulating blood lipids, particularly cholesterol, thus playing a preventive action against atherosclerosis (Capella et al., 2005).

Total polar compounds (TPC) and smoke point

Table 4 shows the data obtained for both total polar compounds (TPC) and smoke point. TPC is a very important chemical parameter, as it is considered the best indicator of the presence of degradation products in frying oil (Marquéz-Ruiz et al., 1996; Bansal et al., 2010). TPC is, in fact, the only parameters included in the EC regulation for the evaluation of frying oils quality; if the TPC content of the frying oil is $\geq$ 25%, it should be discarded. In the present study, TPC were determined by means of the Testo 265 instrument, which contains a sensor that allows a fast evaluation of such molecules. In general, the 70% of frying oil blends here studied had a TPC

around 17%. HOSO/RO/SO and SO/HO exhibited the lowest TPC content (9.1%), while PO/SO and PO/SBO/SO displayed the highest levels (16.9% and 17.3%, respectively). In this last oils, the percentage of TPC detected is highest in comparison with the other oils blends. This characteristic can be attributed to the presence of palm oil among the ingredients of the frying blends. Palm oil has a high percentage of DAG (7-13%) (Gee., 2007) which slightly decrease after refining (1%) and can thus greatly affect oil crystallization and color during storage (Siew et al., 2000; Puah et al., 2007). Moreover, DAG are found to represent almost all of the polar fraction, together with PTG and OTG (Bansal et al., 2010; Nagendran et al., 2000; Marquéz-Ruiz et al., 1996).

Regarding the smoke point, it ranged from 223 to 252°C (Table 4). SO/HO showed the highest smoke point (252°C), while SOHO/SO displayed the lowest one (224°C). The 85% of commercial oils analyzed in the present study showed a smoke point ≤ 231°C; this is a very important feature as the smoke point is generally related to the FA composition of the oils, being lower in those oils with a higher level of short and medium chain FA and a higher degree of unsaturation (Franke, 1980). In fact, the smoke point was higher in those blends with a relevant percentage of MUFA (60-80%) and SFA (23-30%).

Hydrolytic parameters

To assess the initial hydrolytic status of the frying oil blends, both free acidity value and total diacylglycerol (DAG) content were determined. The free acidity value of the blends varied from 0.08 to 0.11% of oleic acid (Table 4), except for SO/HO whose free acidity was equal to 0.4% of oleic acid as it was obtained by biological refining where the value are agree with the refining oil where the limit of FFA is 0.5% (Stazione Sperimentale per le Industrie degli Oli e dei Grassi, 2002)

The determination of total DAG in vegetable oils is a very important parameter, since they are practically not eliminated by the refining processes and, therefore, they provide a real assessment of the hydrolytic status of the vegetable oils. It is important to remember that the amount of diacylglycerol is an important parameters to detect in a frying system, because they can act like a tensioactive molecules, leading to an increase of the surface tension of the frying system. The high interfacial tension in the frying system breaks steam bubbles and forms a steam blanket over the oil surface. The steam blanket reduces the contact between the oil and oxygen, and lowers the oil oxidation (Choe & Min, 2007; Blumenthal, 1991).

Table 5 shows the total DAG content of the frying oil blends and their distribution into the three DAG characteristic groups (D32 (C16-C16 DAG), D34 (C16-C18 DAG) and D36 (C18-C18 DAG)). DAG are biosynthetic intermediates and hydrolytic metabolites of triacylglycerols. The oil blends that had the higher DAG content were those formulated with palm oil; indeed, PO/SO and PO/SBO/SO showed the highest DAG level (4.8%), followed by SO/SBO/PO (2.8%). In these three blends, the D34 constituted the most representative fraction (49.3% vs. 52.9% vs. 44.2%, respectively), which is related to their total FA composition as it is dominated by the presence of palmitic and oleic acids. As already mentioned, DAG are considered an undesirable fraction in palm oil as they affect its crystallization and color upon storage (Gee, 2007; Siew, 2000); moreover, since DAG constitute, together with oxidized triglycerides and polymer of triacylglycerides, the largest fraction of polar compounds, a fresh frying oil with a higher DAG initial content will reach faster the maximum level recommended for polar compounds.

SO/PeO, HOSO/SO, SO/HO and HOSO/RO/SO exhibited the lowest DAG content (1.1% vs. 1.2% vs. 1.8%); such quantitative differences are mainly ascribable to the type of oil used for the blend formulation, since they do not have palm oil among their constituents. In addition, these blends are characterized by the absence of D32, as they are mainly composed by FA with 18 carbon atoms (such as oleic and linoleic acids) (see Table 5).

Oxidative parameters

The oxidative stability of vegetable oils is one of the most important indicators of their quality, as lipid oxidation mainly occurs through a chain-reaction mechanism, which generates several compounds that can greatly modify their sensory and nutritional profile. Table 4 shows the OSI times of the frying oil blends, which ranged from 7 to 22 h. PO/SO and SO/HO displayed the highest OSI values (22.7 ± 0.2 vs. 14.1 ± 1.4, respectively), which are greatly related to their high MUFA content (63.4% and 80.2%, respectively), since it is the most abundant FA class in these oil blends; this trend is confirmed by the fact that their OSI time was inversely related to the unsaturation index

Tocopherols and tocotrienols are abundant in vegetable oils and are well-known for their antioxidant activity, which should protect them from thermo-oxidation (Karabulut et al., 2005; Giannazza et al., 2001; Gordon, 1990; Hoffman, 1989). Natural tocopherols and tocotrienols are retained at considerable levels in finished refined vegetable oils (Karabulut et al., 2005; Simonne et al., 1998).

The OSI time of HOSO/RO/SO and PO/SBO/SO were close to 11 h (10.8 ± 0.2 and 11.5 ± 0.2 h, respectively). HOSO/SO, SO/PeO and SO/SBO/PO had the lowest OSI value (7.1 ± 0.0, 7.5 ± 0.2, and 8.1 ± 0.1, respectively), which could be related to their PUFA level (47.8%, 55.5% and 52.4%, respectively), since it was the most representative FA class in these oil blends.

Regarding the peroxide value (PV), all frying oil blends had PV lower than the PV limit fixed for the single refined oils that constituted the blends (Stazione Sperimentale per le Industrie degli Oli e dei Grassi, 2002). In fact, most frying oil blends had a PV < 2.0 meq O_2/Kg.

As described before, different PV limits are fixed for the refined vegetable oils, since they have different fatty acid compositions and antioxidant levels and they are subjected to diverse extraction

and refining conditions, which can influence the final POV level. However, it is important to remember that oil storage conditions, before and after bottling, may further promote oil oxidation, with a consequent increase on PV. Although the latter is widely used as an indicator of oxidative degradation in vegetable oils, peroxides are very unstable and they are easily converted into secondary oxidation products; therefore, it is advisable to utilize other analytical methods (such as as Kreiss Index and p-Anisidine value) as well, so as to provide a more complete and accurate picture of the oxidative status and stability of the oil.

However, all oil blends were purchased in a local supermarket and it is very important to considering other factors (such as the production steps, packaging and storage conditions) play a fundamental role to detect the quality of some parameters that was investigated in the present study that define the initial quality of the oils.

Conclusions

All frying blends here evaluated exhibited a good hydrolytic and oxidative characteristics. PO/SO and SO/HO had the highest OSI value, which could be related to the elevated percentage of MUFA. The free acidity value of all frying oils was below 0.5%, which is characteristic of refined oils. PO/SO and PO/BO/SO blends displayed the highest DAG percentage; which is probably due to the extraction technology of palm oil as it was used for the formulation of these frying oils. The smoke points were all above 220°C, which is related to their FA composition. The TPC detected in the frying oils blend here studied had a TPC more higher in the blend that are formulated with palm olein where the high percentage of DAG where the their presence plays a decisive role for the specific percentage detected. It must be, however, underlined that the performance of these oil blends during frying will greatly depend on the combination of the attributes and characteristics of

the frying oils (FA composition, DAG content and initial oxidative status), which will directly affect their oxidative stability, foam production and polar compounds content.

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Table 1. Single vegetable oils that constitute the blends and their corresponding code.

CRUDE FRYING OILS COMPOSITION	**CODE**
Sunflower oil, soybean oil, palm oil	SO/SBO/PO
High-sunflower oil, sunflower oil	SOHO/SO
Sunflower oil, hazelnut oil	SO/HO
Sunflower oil, peanut oil	SO/PeO
Palm oil, soybean oil, sunflower oil	PO/SBO/SO
High-sunflower oil, rapeseed oil, sunflower oil	HOSO/RO/SO
Palm oil, sunflower oil	PO/SO

Table 2. Main fatty acids (expressed as % of total FA) present in the fresh commercial frying oils

	SO/SBO/PO	HOSO/SO	SO/HO	SO/PeO	PO/SBO/SO	HOSO/RO/SO	PO/SO
C16:0	12.9 ± 0.2	5.3 ± 0.0	4.2 ± 0.1	5.7 ± 0.1	25.2 ± 0.6	4.5 ± 0.2	17.7 ± 0.0
C18:0	3.2 ±0.0	3.2 ± 0.0	3.1 ± 0.0	3.2 ± 0.0	3.3 ± 0.1	2.7 ± 0.0	3.4 ± 0.0
C18:1	29.6 ± 0.1	42.1 ± 0.0	79.8 ± 0.2	33.7 ± 0.1	33.7 ± 0.3	60.5 ± 0.0	63.0 ± 0.2
C18:2	49.6 ± 0.2	47.5 ± 0.1	10.7 ± 0.0	55.2 ± 0.0	32.6 ± 0.3	26.2 ± 0.0	13.4 ± 0.1
C18:3	2.7 ± 0.0	0.1 ± 0.1	0.3 ± 0.0	0.1 ± 0.1	2.6 ± 0.0	4.0 ± 0.1	0.2 ± 0.0
Others	2.0 ± 0.1	1.8 ± 0.0	1.9 ± 0.1	2.1 ± 0.1	2.5 ± 0.0	2.2 ± 0.1	2.2 ± 0.1

Data are presented as mean values ± standard deviation (SD). Mean values with the same letter are not different ($P \leq 0.05$).

Table 3 FA classes detected in the fresh frying oils blend

	SO/SBO/PO	HOSO/SO	SO/HO	SO/PeO	PO/SBO/SO	HOSO/RO/SO	PO/SO
[a]SFA	15.6 ± 0.6	9.4 ± 0.3	8.3 ± 0.4	10.0 ± 0.3	27.2 ± 1.4	6.5 ± 0.4	21.0 ± 0.8
[a]MUFA	26.8 ± 0.5	40.5 ± 1.4	76.1 ± 2.8	32.6 ± 1.3	30.8 ± 2.4	47.7 ± 2.1	58.3 ± 2.5
[a]PUFA	46.7 ± 0.5	45.6 ± 1.7	10.4 ± 0.6	53.0 ± 1.9	32.0 ± 2.5	23.5 ± 1.0	12.6 ± 0.4
[a]SFA/PUFA	0.3	0.2	0.8	0.2	0.9	0.3	1.7
[b]1/unsaturation	533	507	191	574	402	320	207

[a] Data are presented as mean values ± standard deviation (SD). Mean values with the same letter are not different ($P \leq 0.05$).

[b] It's has been calculated by multipling the relative percentage of different types of unsaturated fatty acids by a factor different from the predeterminated oxidative stabilitty of each unsaturated fatty acid. The factors are 1 for momounsaturated, 10 and 20 for di and triinsatured

Table 4. Physico-chemical parameters determined in fresh frying oil blends

	SO/SBO/PO	**HOSO/SO**	**SO/HO**	**SO/PeO**	**PO/SBO/SO**	**HOSO/RO/SO**	**PO/SO**
FFA (%oleic acid)	0.1 ± 0.01	0.1 ± 0.00	0.4 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.01	0.1 ± 0.01
[a]OSI (h)	8.1 ± 0.1	7.1 ± 0.0	14.1 ± 1.8	7.5 ± 0.2	11.5 ± 0.4	10.8 ± 0.2	25.3 ± 0.1
PV (meq O2/kg of oil)	1.6 ± 0.0	5.5 ± 0.2	3.1 ± 0.1	1.3 ± 0.1	1.5 ± 0.0	0.9 ± 0.1	3.7 ± 0.1
TPM (%)	16.9 ± 0.1	15.6 ± 0.1	9.1 ± 0.1	15.9 ± 0.1	16.9 ± 0.2	9.1 ± 0.0	17.3 ± 0.2
Smoke Point (°C)	232 ± 2.0	224 ± 2.0	252 ± 2.0	232 ± 2.0	231 ± 1.0	223 ± 3.0	231 ± 2.0

[a] Data are presented as mean values ± standard deviation (SD). Mean values with the same letter are not different ($P \leq 0.05$).

Table 5. Total DAG content (%) and DAG distribution (%) in different classes.

	DAG (%)	DAG 32 (%)	DAG 34 (%)	DAG 36 (%)
SO/SBO/PO	2.8 ± 0.1	5.1 ± 0.1	44.2 ± 0.1	50.7 ± 0.2
HOSO/SO	1.2 ± 0.0	0.0 ± 0.0	14.7 ± 1.0	85.3 ± 1.0
SO/HO	1.8 ± 0.2	0.0 ± 0.0	9.4 ± 0.3	90.6 ± 0.3
SO/PeO	1.1 ± 0.0	0.0 ± 0.0	15.2 ± 0.1	84.8 ± 0.1
PO/SBO/SO	4.8 ± 0.3	5.7 ± 0.1	52.9 ± 0.1	41.3 ± 0.0
HOSO/RO/SO	3.0 ± 0.1	0.0 ± 0.0	8.3 ± 0.2	91.7 ± 0.2
PO/SO	4.8 ± 0.1	5.5 ± 0.1	49.3 ± 0.3	45.2 ± 0.2

[a] Data are presented as mean values ± standard deviation (SD). Mean values with the same letter are not different ($P \leq 0.05$).
Total DAG content (%) and distribution of the DAG classes (%)

Chemical Performance of Different Vegetable Oils During Frying Under Standardized Lab-Scale and Real Catering Conditions

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Abstract

The aim of this work was to compare the oxidative and hydrolytic stability of a vegetable oil blend (high-oleic sunflower oil:palm olein, 60:40, v/v) with that of palm olein, during frying under standardized lab-scale and real catering conditions. Firstly, both vegetable oils were utilized for French fries frying under standardized lab-scale conditions. In a second phase, the performance of both vegetable oils was tested in two catering situations (restaurant and cafeteria), where different food products (potatoes, battered vegetables, chicken nuggets and floured fish) and quantities were fried depending on the customer's request. In general, the oxidative stability index (OSI) decreased during the frying process in the three tested conditions, whereas the peroxide value (PV) showed an increase (≤18 meq O_2/kg oil) followed by the classical sinusoidal behaviour as a consequence of the simultaneous formation of PV and its degradation into secondary oxidation products. Both vegetable oils showed a good oxidative stability under standardized lab-scale conditions; however, their performance in the restaurant was completely different, which was mainly related to the type and quantity of fried food. Regarding the hydrolytic stability, the free acidity rose during frying (< 0.4% oleic acid) in all three tested conditions. Total diacylglycerol (DAG) content remained almost constant, being lower in the frying oil blend (≤ 5%) than in palm olein (≤ 12%);the latter, in fact, reflects the influence of the extraction technology of palm olein on its DAG content.

Key words: deep frying, vegetable oilsoxidation lipolysis, standardized frying conditions, catering frying ,.

Introduction

Frying is one of most important and popular procedures for food preparation used worldwide. This is probably due to the peculiar characteristics of fried products, such as color, flavor and texture, which make them very attractive and tasty to the palate. Deep frying is a very fast and cheap cooking method and, for this reason, it is involved and used at different levels, such as catering, snack food industries and for the production of pre-cooked refrigerated or frozen food.

Consumption of deep-fried food has strongly increased in the last decades, especially since the consumption of frozen food has become more and more important and fast food has turned into a growing market (Matthaus, 2006). The economic impact of the frying industry amounts to about $ 83 billion of U.S. dollars and it is almost double for the rest of the world (Pedreschi et al., 2005). Frying implies food immersion in an oil bath kept at high temperatures (between 160°C and 180°C), in contact with air (Gertz, 2000). This type of food processing operation causes a series of physico-chemical modifications in both oil and food, which depend on the nature of the oil, the type of food, the amount of food to be fried and the processing conditions (temperature, time, holding conditions, oil exchange). More than 500 different chemical compounds formed as a result of autoxidation, thermoxidation, pyrolysis and polymerization, have been detected in used frying oils (Gertz, 2004). These modifications can generate a series of chemical compounds with adverse nutritional implications and potentially hazardous effects for human health (Marquez-Ruiz et al., 1996). Thermoxidation, hydrolysis, and polymerization are the main reactions that occur during frying, which induce a gradual decrease of the initial oil quality. The high temperatures reached by the oil are among the main causes of oxidative rancidity of fats in frying baths. However, hydrolysis is the major chemical reaction that takes place during deep-oil frying; this degradation process leads to triglyceride lysis into free fatty acids (FFAs), monoacylglycerols, diacylglycerols (DAG) and glycerol. During frying, when heat and water are present, FFAs may develop rapidly if poor processing techniques are adopted.. It is evident, therefore, that the control of chemical and physical

processes during frying turns out to be quite difficult. In addition, there are other variables that must be considered and controlled, such as frying mode (continuous or discontinuous), surface-to-oil volume ratio, temperature, oil unsaturation degree, and presence of naturally occurring or added minor compounds. All the aforementioned phenomena lead to a change in the quality of used oils and food fried therein. Recently, the fast-food industry is adopting various methods to maintain the quality and to increase the useful life of frying oils. In fact, determining the time when frying oils reach their maximum safe levels of deterioration is a challenging task (Paul et al., 1997), especially when it is applied to real catering conditions (small and large restaurants and fast food eating places).

The purpose of this work was to compare the oxidative and hydrolytic stability of a vegetable oil blend (high-oleic sunflower oil:palm olein, 60:40, v/v) with that of palm olein, during frying under standardized lab-scale and real catering conditions. Firstly, both vegetable oils were utilized for French fries frying under standardized lab-scale conditions (Temperature 180°C; continuos condition). In a second phase, the performance of both vegetable oils was tested in two catering situations (restaurant and cafeteria), where different food products (potatoes, battered vegetables, chicken nuggets and floured fish) and quantities were fried depending on the customer's request.

Materials and Methods

2.1 Samples

High-oleic sunflower oil and palm olein were provided by a local oil bottling company (Italy). A high-oleic sunflower oil:palm olein blend (60:40, v/v) was prepared with oils from the same production batch.

2.2 Experimental conditions for the frying trials

The frying performance was first evaluated in lab-scale conditions and afterwards in two real catering conditions (restaurant and cafeteria).

2.2.1 Frying procedure in the lab-scale conditions

Deep frying experiments were carried out using a stainless electrical fryer (FD 50, Roller Grill, Bonneval, France) with 5 L capacity. Prefried potato sticks, purchased at a local store (Bologna), were used for these trials; the potato sticks belonged to the same batch and, as declared in the label, were prefried with palm olein by the producer. Table 1 describes the frying experimental conditions and sampling. Briefly, about 4.6 L of oil were heated at 180°C and allowed to equilibrate at this temperature for about 10 min. About 150 g of prefried potato sticks were intermittently fried for 4 min, at 5-min intervals; oil sampling (75 mL) was carried out. A total of 3 Kg of potato sticks were fried in 190 min of deep frying. All samples were stored at -18°C until the analysis.

2.2.2 Frying procedure in the restaurant conditions

Deep frying experiments were performed using a professional stainless steel open fryer with 25 L of capacity and equipped with 2 stainless steel baskets. French fries, battered vegetables, and chicken nuggets were fried depending on the consumer's request. Table 1 describes the frying experimental conditions and sampling. Briefly, about 25 L of oil were introduced into the fryer, heated at 175°C and allowed to equilibrate at this temperature for 10 min. The oil was heated 9 h/day (4 h on the morning and 5 h in the evening) for 3 consecutive days. The fryer was turned off at the end of every frying step in the morning and in the evening. Oil sampling (75 mL) was carried out before and after frying in the morning and after frying in the evening. All samples were stored at -18°C until the analysis.

2.2.3 Frying procedure in the cafeteria conditions

Deep frying trials were performed using a professional stainless steel open fryer with 25L of capacity and equipped with 1 stainless steel basket. French fries, battered vegetables, chicken nuggets and floured fish were fried depending on the consumer's request. Table 1 describes the frying experimental conditions and sampling. Briefly, about 25 L of oil were introduced into the fryer, heated at 180°C and allowed to equilibrate at this temperature for 10 min. The whole experiment lasted 12 days (two weeks), but the fryer worked for 4 non-consecutive days for the set of trials for each frying oil. The oil was heated for about 4 h when frying was carried out. The fryer was turned off at the end of every frying step in the morning, as well as in the non-frying days. The oils was replenished with fresh oil (about 3L) at the 5th and the 3rd frying days of the oil blend and palm olein frying experiments, respectively. Oil sampling (75 mL) was carried out before and after frying in the morning and after frying in the evening. All samples were stored at -18°C until the analysis.

2.3 Reagents, solvents, and standards

Diethyl ether, double distilled water, *n*-hexane, chloroform, isopropanol, methanol, glacial acetic acid, phenolphtalein, sodium thiosulfate and starch water, were supplied by Carlo Erba Reagenti (Milano, Italy). Potassium iodure was supplied by Sigma-Aldrich (St. Louis, MO, USA). Anhydrous sodium sulfate was purchased from BDH (BDH, England). Commercial standards of diacylglycerols (dimyristin, dipalmitin, distearin, diolein) and 5α-cholestan-3β-ol (dihydrocholesterol used as internal standard for the quantification of diacylglycerols) were supplied by Sigma Aldrich (St. Louis, MO, USA). Silica solid phase-extraction (SPE) cartridges NH_2 (500 mg stationary phase/3 mL) were purchased from Phenomenex (Torrence, CA, USA). The silylation mixture was prepared with dried pyridine (ACROS Organics, USA), hexamethyldisilazane (BDH, England) and trimethylchlorosilane (Carlo Erba Reagenti, Milan, Italy) at a ratio of 5:2:1 by volume.

2.4 Determination of oxidative stability

2.4.1 Oxidative Stability Index (OSI) of frying oils

Oxidative stability of samples were determined according to the AOCS Official Method Cd12b.92 (AOCS Official Method Cd 12b-92). The analysis were carried out by the Omnion Oxidative Stability Instrument (OSI) with eight channels (Omnion, IL). Frying oil (5.0 ± 0.1 g) was placed in a polycarbonate tube and heated at 110±1°C under atmospheric pressure and at 150 mL/min of air flow rate. This test is based on the increase in conductivity due to the formation of volatile acids (mainly formic acid) during oil accelerated oxidation (Jebe et al., 1993). The conductivity was measured in polycarbonate tubes using twice distilled water. Results were expressed as induction time (h). Two replicates were analyzed per sample.

2.4.2 Determination of peroxide value (PV)

Peroxide value (PV) (expressed as meq O_2/kg oil) was evaluated according to the official method described in annex III of EEC Regulation 2568/91 (EC Commission Regulation EEC No. 2568/91 of July 1991). About 5.0 ± 0.1 g of oil samples were dissolved in a glacial acid acetic:chloroform (3:2, v/v) solution, added with 0.5 mL of potassium iodure, stirred for 1 min and then stored for 5 min at darkness. Afterwards, the solution was diluted with 75 mL of bidistilled water and titrated with sodium thiosulfate 0.01 N. Two replicates were analyzed per sample.

2.5 Hydrolytical stability

2.5.1 Determination of free acidity (FFA)

Free acidity value (expressed as % oleic acid) was evaluated according to the official method described in annex III of EC Regulation 2568/91 (EC Commission Regulation EEC No. 2568/91 of July 1991). Two replicates were analyzed per sample.

2.5.2 Determination of total diacylglycerol (DAG) content

DAG were determined according to a modified version of the method suggested by Bonoli et al. (Bonoli et al., 2007), where dihydrocholesterol was used as internal standard. Seventy µL of a solution of dihydrocholesterol (1.052 mg of dihydrocholesterol in 1 mL of *n*-hexane:isopropanol (4:1, v/v)) were added to 100 mg oil and dissolved in 500 µL of *n*-hexane before loading into SPE. The rest of DAG purification by SPE elution was the same as reported by Bortolomeazzi et al. (1990). The purified fraction was then silylated (Sweeley et al., 1963), dried under nitrogen stream and dissolved in 100 μL of *n*-hexane. One microliter of the silylated solution was injected into a gas chromatograph (GC 8000 Series Fisons Instruments, Milano, Italia) with injector spit-splittless and

a flame ionization detector (FID). A fused silica capillary column (25 m x 0.25 mm i.d. x 0.1 µm of film thickness) coated with 65% diphenyl-polysiloxane–35% dimethyl-polysiloxane (TAP, Varian, Lake Forest, USA), was used. The oven temperature was programmed from 160°C to 350°C at 3°/min and kept a 350°C for 20 min. The injector and detector temperatures were set at 350°C. Helium was used as carrier gas at a flow of 1.40 mL/min (pressure, 80 kPa); the split ratio was 1:30. Total DAG content of the samples was expressed as percentage. Two replicates were analyzed per sample.

Determination of total polar compounds (TPM)

Total polar compounds were measured by Testo 265 (electronic cooking oil tester) (CP B 04/11/512 (PRO 04/11/015). The sensor based on parallel plate capacitor was immersed in the hot oil at frying temperature for about 5 min, after completion of the frying cycles. The TPC and temperature readings were carried out once the sensor had stabilized (around 1 min). The Testo 265 instrument provided a TPC value (expressed as %), with a $\pm$ 2% of standard deviation of the instrumental measurement. Two replicates were analyzed per sample.

Determination of the smoke point

This determination was performed according to the NGD C77 method (NGD C77, 1976), by using a Cleveland instrument. The smoke point is directly correlated to the fatty acid composition of the oil; in fact, lower smoke points are generally measured in oils and fats with a high content of PUFA, due to the lower stability of the double bonds at high temperatures. The smoke point, therefore, is an important determination to assess the degradation level of an oil/fat when subjected to heating. Briefly, the oil is placed in an brass container, up to the knurling fixed level. The oil is heated and, when it starts smoking, the temperature is registered. Two replicates were analyzed per sample.

Statistical analysis

The data are reported as mean values of 2 independent replicates (*n*= 2) of each analytical determination. One-way ANOVA was performed, in order to compare data obtained for different frying times and different oils.

3. Results

Monitoring of chemical parameters in lab-scale conditions

The oxidative stability of vegetable oils is one of the most important indicators of their quality, as lipid oxidation mainly occurs through a chain-reaction mechanism, which generates several compounds that can greatly modify their sensory and nutritional profile. According to Yavary et al. (2010), the evaluation of OSI time on crude vegetable oils (by Rancimat test or OSI instrument) cannot guarantee or predict the actual frying performance of the oil, but it can be considered a useful "screening" test, to reduce the possibility of introducing low stability oils into the production area (Morton et al., 1988).

Table 2 shows the OSI times of the fresh oils and samples collected during the lab-scale condition. Significant differences were detected in the OSI times of the two frying oils. The oils actually exhibited resistance to oxidative stress caused by frying; in fact, the OSI time reduction detected in the blend was about 74%, whereas it was about 76% in the palm olein.

Primary oxidation products were evaluated by the determination of peroxide value (PV). Since PV is related to oxidative rancidity, its determination is essential to assess the lipid quality and, in fact, is one of the most widely used methods for testing the quality of fats and oils. However, it is not exhaustive, since peroxides are highly unstable, so they easily decompose and convert into other oxidation products. Although a linear correlation has been found between PV and the levels of

odors during the initial stages of lipid oxidation (O'Brien, 1998), this method alone is not a good indicator for the oil's sensory quality. This is because PV increases up to a maximum level and then decreases as storage time increases. In any case, a high PV indicates generally low sensory quality (present or future), but a low PV is not always an indicator of good quality.

In the present study, all fresh oils had a PV lower than the PV limit fixed for the refined oils that constituted the blend (0.07 meq O_2/kg of oil) (Stazione Sperimentale per le Industrie degli Oli e dei Grassi, 2002). In both oils, it was observed an increase of PV reaching a maximum of 4.5 meq O_2/kg of oil. As reflected by such trends, peroxides are unstable under frying conditions and are formed with different velocities depending on their FA composition and unsaturation degree. In general, an increase in the peroxide value during the initial frying step would be expected to be followed by a decrease after further frying, because hydroperoxides tend to decompose at 180°C to form secondary oxidation products.

The free acidity value detected in the fresh oil blend and palm olein was very low (0.07% of oleic acid), as expected for refined oils. A gradual increase of free acidity was detected in both oils during frying day, being more intense in palm olein. These trends can be related to the higher percentage of diacylglycerols present in the palm olein (Table 3), as they are more prone to hydrolysis as compared to triacylglycerols.

Table 3 shows the total diacylglycerol (DAG) content of the frying oils and their distribution into the three DAG characteristic groups (D32 (C16-C16 DAG), D34 (C16-C18 DAG) and D36 (C18-C18 DAG)). It's important to remember that the amount of diacylglycerol is an important parameters to detect in a frying system because their can act like a tensioactive molecules leading to increase the surface tension of frying system. The high interfacial tension in the frying system breaks steam bubbles and forms a steam blanket over the oil surface. The steam blanket reduces the contact between the oil and oxygen, and lowers the oil oxidation (Choe & Min, 2007; Blumenthal 1991). The DAG level of palm olein (11.6%) was higher than that of the oil blend (4.5%).

Dyacylglycerols are not affected by the refining processes these component are hydrolytic metabolites and also biosynthetic intermediates of tryacylglycerols. Dyacylglycerols are considered undesiderable in palm oil as they affect crystallization and colority of palm olein upon storage (Gee, 2007; Siew, 2000).

The dyacylglycerol together oxidized triglycerides and polymer of tryglicerid concour to form the largest part of polar compounds. It's clear that a fresh frying oil with a high content in diacylglycerol will be subject to more quickly to reach the maximum level recommended for polar compounds.

The D_{34} was the most representative DAG fraction in both frying oils (53% vs 58% in the blend and palm olein, respectively), followed by D_{32} (7% and 6% in the blend and palm olein, respectively), and D_{36} group representing 45% and 36% of total DAG in the blend and palm olein, respectively. In general, no significant changes in DAG content was found during the different number of frying cycles. The determination of total DAG in vegetable oils is a very important parameter, since they are not eliminated by the refining processes and, therefore, they provide a real assessment of the hydrolytic status of the vegetable oils. In addition, during frying, DAG act as surfactant molecules, leading to increase the surface tension of the frying system. The high interfacial tension in the frying system breaks steam bubbles and forms a steam blanket over the oil surface. The steam blanket reduces the contact between the oil and oxygen, and lowers the oil oxidation (Choe & Min, 2007; Blumenthal, 1991).

The smoke point of the fresh oil blend and palm olein was very similar (254°C vs. 248°C, respectively). These values are higher than those reported for most refined edible oils (180-230°C) (Matthaus, 2010). FFA and other volatile substances affect the smoke point. Although the determination of the smoke point is not an important indicator for defining the performance of

frying oils, it can provide a suggestion of the maximum temperature up to which the oil can be heated. Morton and Chidley (1988) reported that the amount of smoke emanating from a cup is directly proportional to the concentration of low molecular weight decomposition products in the oil. However, the determination of smoke point through a standard procedure still heavily relies on the ability of the worker to determine the point at which the oil begins to smoke (Stevenson, 1984).

Monitoring of chemical parameters in real restaurant conditions

Table 4 shows the OSI times of the fresh oils and samples collected during the discontinuous frying steps. The OSI time reduction detected in the blend was more highest than palm olein (90% vs. 25%). The low polyunsaturated fatty acid content and high levels of antioxidants in palm oil provide good oxidative stability whereas the preferential enrichment of oleic and linoleic acids in the *sn*-2 position provides better bioavailabilty of oleic acid as monounsaturated fatty acid and linoleic acid as essential fatty acid, as compared to oils and fats of similar composition but with randomized fatty acid distribution (Gee, 2007). Inoltre, the contribution of natural antioxidants to the oxidative stability of oils should not be overlooked; commercial red palm olein was found to contain almost equal amounts of α- and β-carotene of crude palm oil (Bonnie et al., 1999). The provitamin A efficacy of red palm oil has been documented by a number of investigators. Apart from this major nutritional implication, carotenoids have significant antioxidant properties. Both α- and β-carotene, as well as lycopene, are important antioxidants because of their ability to act as effective quenchers of singlet oxygen (Dimascio et al., 1989). Palm carotenoids have also been suggested to have possible inhibitory effects on the development of certain types of cancer. Their role in inhibiting the proliferation of several types of cancers, such as oral, pharyngeal, lung, and stomach cancers, has been investigated (Murakoshi et al. 1992). The palm olein displayed a higher total tocopherol content than the blend where it was probably not enough to further attenuate the

oxidative process in this system. Tocopherols and tocotrienols are abundant in vegetable oils and are well-known for their antioxidant activity, which should protect them from thermo-oxidation (Karabulut et al., 2005; Giannazza et al., 2001; Gordon, 1990; Hoffman, 1989). Natural tocopherols and tocotrienols are retained at considerable levels in finished refined vegetable oils (Karabulut et al., 2005; Simonne et al., 1998). Due to a foaming phenomena, it was not possible to record the OSI time value during the second frying day.

Primary oxidation products were evaluated by the determination of peroxide value (PV). Since PV is related to oxidative rancidity, its determination is essential to assess the lipid quality and, in fact, is one of the most widely used methods for testing the quality of fats and oils. However, it is not exhaustive, since peroxides are highly unstable, so they easily decompose and convert into other oxidation products.

In the present study, the fresh oil blend had a PV lower than the PV limit fixed for the refined oils that constituted the blend (0.07 meq O_2/kg of oil) (Stazione Sperimentale per le Industrie degli Oli e dei Grassi, 2002). The fresh palm olein showed more highest PV 2.71 meq O_2/kg of oil. In both oils, it was observed an increase of PV with a sinusoidal trend, reaching a maximum of 16.8 meq O_2/kg of oil in the blend at the third frying day (Table 4) and 9.5 meq O_2/kg of oil in the palm olein at the second frying day (Table 4). As reflected by such trends, peroxides are unstable under frying conditions and are formed with different velocities depending on their FA composition and unsaturation degree. In general, an increase in the peroxide value during the initial frying step would be expected to be followed by a decrease after further frying, because hydroperoxides tend to decompose at 180°C to form secondary oxidation products. The overall increase of peroxide values occurred particularly during the cooling period, where the frying oil is exposed to air at low temperature. In this experimental conditions, the oil has been subjected to a high oxidative stress being subjected to thermal shock during the day and also subjected to cooking of the amount of

food according to the requirements of the room which have probably helped to promote the oxidation.

The free acidity value detected in the fresh oil blend and palm olein was very low (0.07% of oleic acid), as expected for refined oils. A gradual increase of free acidity was detected in both oils during frying day, being more intense in palm olein. These trends can be related to the higher percentage of diacylglycerols present in the palm olein (Table 5), as they are more prone to hydrolysis as compared to triacylglycerols.

Table 5 shows the total diacylglycerol (DAG) content of the frying oils and their distribution into the three DAG characteristic groups (D_{32} (C16-C16 DAG), D_{34} (C16-C18 DAG) and D_{36} (C18-C18 DAG)). The DAG level of palm olein (11.5%) was higher than that of the oil blend (4.2%). Total DAG level is of outmost importance, since it could have a noticeable effect on foam formation and, thus, affect frying oil performance. The D_{34} was the most representative DAG fraction in both frying oils (48% vs 59% in the blend and palm olein, respectively), followed by D_{36} group with 45% and 36% of total DAG in the blend and palm olein, respectively. The D_{32} was present at low percentages in these oils (5% and 7% in the blend and palm olein, respectively). In general, no significant changes in DAG content was found during the different number of frying cycles. The determination of total DAG in vegetable oils is a very important parameter, since they are not eliminated by the refining processes and, therefore, they provide a real assessment of the hydrolytic status of the vegetable oils.

The smoke point of the fresh oil blend and palm olein was very similar (254°C vs. 247°C, respectively). These values are higher than those reported for most refined edible oils (180-230°C) (Matthaus, 2010). FFA and other volatile substances affect the smoke point. Although the determination of the smoke point is not an important indicator for defining the performance of frying oils, it can provide a suggestion of the maximum temperature up to which the oil can be

heated. Morton and Chidley (1988) reported that the amount of smoke emanating from a cup is directly proportional to the concentration of low molecular weight decomposition products in the oil. However, the determination of smoke point through a standard procedure still heavily relies on the ability of the worker to determine the point at which the oil begins to smoke (Stevenson, 1984).

TPM were determinate by Testo 265, which provided a TPM measurement expressed as percentage. A sensor based on parallel plate capacitor was immersed in hot oil at the fixed frying temperature for about 5 min, after the completion of the designated frying cycles. The Graph 1 report the TPM value detected in the present study. As evinced in the graphs, the fresh frying oils already presented a different TPM level before being subjected to the thermal treatment (10.0% and 15.0% for the oil blend and palm olein, respectively). This difference could be attributed to the different nature of the oils as related to their chemical composition; in fact, most TPM detected are ascribable to the presence of a higher DAG percentage in palm olein, since these compounds are not removed during the refining process (Gee, 2007). During the frying-day, the TPM value ranged from 10.0% to 22.5% in the blend and from 15.0% to 20.5% in the palm olein. In the latter, the percentage of TPM is lower than the maximum level suggested by the Italian Regulation (CM, 1991).

Monitoring of chemical parameters in real cafeteria conditions

Table 6 shows the OSI times of the fresh oils and samples collected during the discontinuous frying steps. Significant differences were detected in the OSI times of the two frying oils. The oils actually exhibited different resistance to oxidative stress caused by frying and by not standardized frying operations; in fact, the OSI time reduction detected in the blend was about 58%, whereas it was about 45% in the palm olein. It's interesting to note like in the last two step of sampling for both frying oils was detected an increase of OSI time that's ranged from 14.5 h to 18.3 h in the blend and

from 10.4 h to 16.4 h in the palm olein. In the last frying-days the OSI time increase might be due to the formation of Maillard reaction products that derived from floured fish frying.

Primary oxidation products were evaluated by the determination of peroxide value (PV). Since PV is related to oxidative rancidity, its determination is essential to assess the lipid quality and, in fact, is one of the most widely used methods for testing the quality of fats and oils. However, it is not exhaustive, since peroxides are highly unstable, so they easily decompose and convert into other oxidation products. Although a linear correlation has been found between PV and the levels of odors during the initial stages of lipid oxidation (O'Brien, 1998), this method alone is not a good indicator for the oil's sensory quality. This is because PV increases up to a maximum level and then decreases as storage time increases. In any case, a high PV indicates generally low sensory quality (present or future), but a low PV is not always an indicator of good quality.

In the present study, all fresh oils had a PV highest than the PV limit fixed for the refined oils that constituted the blend (1.5 meq O_2/kg of oil) (Stazione Sperimentale per le Industrie degli Oli e dei Grassi, 2002). In both oils, it was observed an increase of PV with a sinusoidal trend, reaching a maximum of 7.8 meq O_2/kg of oil in the blend at the third frying day (Table 6) and 8.5 meq O_2/kg of oil in the palm olein at the fourth frying day (Table 6). As reflected by such trends, peroxides are unstable under frying conditions and are formed with different velocities. In general, an increase in the peroxide value during the initial frying step would be expected to be followed by a decrease after further frying, because hydroperoxides tend to decompose at 180°C to form secondary oxidation products.

The free acidity value detected in the fresh oil blend and palm olein was very low (0.07% of oleic acid and 0.15% of oleic acid), as expected for refined oils. A gradual increase of free acidity was detected in both oils during frying day, being more intense in palm olein. These trends can be related to the higher percentage of diacylglycerols present in the palm olein (Table 7), as they are more prone to hydrolysis as compared to triacylglycerols.

Table 7 shows the total diacylglycerol (DAG) content of the frying oils and their distribution into the three DAG characteristic groups (D_{32} (C16-C16 DAG), D_{34} (C16-C18 DAG) and D_{36} (C18-C18 DAG)). The DAG level of palm olein (11.1%) was higher than that of the oil blend (4.6%). The D_{32} was present at low percentages in these oils (about 6% in the blend and palm olein, respectively). The D_{34} was the most representative DAG fraction in palm oil (58.5% vs 44.9% detected in the blend), the D_{36} group was the second most abundant one, representing 48.9% in the blend and 35.7% in the palm oil. The determination of total DAG in vegetable oils is a very important parameter, since they are not eliminated by the refining processes and, therefore, they provide a real assessment of the hydrolytic status of the vegetable oils. In addition, during frying, DAG act as surfactant molecules, leading to increase the surface tension of the frying system. The high interfacial tension in the frying system breaks steam bubbles and forms a steam blanket over the oil surface. The steam blanket reduces the contact between the oil and oxygen, and lowers the oil oxidation (Choe & Min, 2007; Blumenthal, 1991).

The smoke point of the fresh oil blend and palm olein was very similar (254°C vs. 247°C, respectively). These values are higher than those reported for most refined edible oils (180-230°C) (Matthaus, 2010). FFA and other volatile substances affect the smoke point. Although the determination of the smoke point is not an important indicator for defining the performance of frying oils, it can provide a suggestion of the maximum temperature up to which the oil can be heated. Morton and Chidley (1988) reported that the amount of smoke emanating from a cup is directly proportional to the concentration of low molecular weight decomposition products in the oil. However, the determination of smoke point through a standard procedure still heavily relies on the ability of the worker to determine the point at which the oil begins to smoke (Stevenson, 1984).

TPM were also determinated by Testo 265, which provided a TPM measurement expressed as percentage. A sensor based on parallel plate capacitor was immersed in hot oil at the fixed frying temperature for about 5 min, after the completion of the designated frying cycles. During the frying

day the TPC ranged from 9.5% to 18% in the blend and from 17% to 40% in the palm oil. In this last case, the percentage of TPC is more higher than the maximum level suggested by the Italian Regulation (CM, 1991). This difference could be attributed to the different nature of the oils as related to their chemical composition; in fact, most TPM detected are ascribable to the presence of a higher DAG percentage in palm olein, since these compounds are not removed during the refining process. And probably is to ascribe at the different frying condition and the different typology of food fried in the bach.

CONCLUSIONS

Under standardized conditions, both vegetable oils showed a good oxidative and hydrolytical stability; the differences observed on the free acidity value and diglyceride content are related to the oils' extraction and refining technology. In the restaurant and cafeteria trials, different oil behaviours were identified during frying, which could be ascribed to the different quantity and/or type of fried food. In these catering conditions, the palm olein displayed a greater oxidative stability than the frying oil blend, which is probably related to the higher content of saturated fatty acids (41%) present in the palm olein as compared to the blend (21%) (data not shown). From the hydrolytic standpoint, the palm olein displayed a diacylglycerol content (11%) that was almost trice as much that of the frying oil blend (4%). However, such diacylglycerol levels remained fairly constant regardless of the frying conditions (time, temperature, amount/type of fried food); this parameter trend is of outmost importance, since diacylglycerols can have a noticeable effect on foam formation and, thus, affect frying oil performance. Considering the overall performance of both oils under standardized lab-scale and real catering conditions, the frying oil blend therefore represents a good alternative to the utilization of palm olein.

Table 2. Oxidative parameters detected on the fresh frying oils and their changes (evolution) during frying in lab-scale conditions.

Frying Time (min)	**BLEND**	**PALM OLEIN**	**BLEND**	**PALM OLEIN**
	OSI (h)		**POV (meqO2/Kg of oil)**	
0	25.3 ± 0.4	25.3 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
14	22.9 ± 0.2	22.7 ± 1.0	3.1 ± 0.2	3.0 ± 0.2
41	22.5 ± 0.4	22.3 ± 0.6	3.0 ± 0.1	3.7 ± 0.3
77	20.6 ± 0.2	21.9 ± 0.8	3.0 ± 0.2	4.2 ± 0.3
95	20.1 ± 0.2	20.6 ± 0.1	3.3 ± 0.1	4.0 ± 0.0
140	18.8 ± 0.1	19.9 ± 0.6	4.5 ± 0.1	3.9 ± 0.2
185	18.6 ± 0.0	19.0 ± 0.2	4.4 ± 0.0	4.0 ± 0.3

[a] Data are presented as mean values ± standard deviation (SD). Mean values with the same letter are not different ($P \leq 0.05$).

Table 3. FFA and DAG content (%) and DAG distribution (%) in different classes detected on the fresh frying oils and their changes (evolution) during frying day in lab-scale condirtions

Frying Time (min)	**BLEND**	**DAG (%)**	**DAG 32 (%)**	**DAG 34 (%)**	**DAG 36 (%)**
	FFA (% of Oleic Acid)				
0	0.07 ± 0.00	4.2 ± 0.2	6.6 ± 0.1	48.0 ± 0.2	45.4 ± 0.3
14	0.07 ± 0.00	4.4 ± 0.4	6.7 ± 0.1	48.3 ± 0.1	45.0 ± 0.0
41	0.10 ± 0.00	5.2 ± 0.5	6.8 ± 0.2	47.9 ± 0.3	45.3 ± 0.5
77	0.11 ± 0.00	4.5 ± 0.1	6.7 ± 0.1	47.7 ± 0.2	45.6 ± 0.3
95	0.11 ± 0.00	4.5 ± 0.4	6.9 ± 0.2	48.2 ± 0.1	44.9 ± 0.2
140	0.11 ± 0.00	4.9 ± 0.1	6.9 ± 0.2	47.4 ± 0.2	45.8 ± 0.2
185	0.12 ± 0.00	4.5 ± 0.2	7.1 ± 0.1	48.1 ± 0.2	44.9 ± 0.2
	PALM OIL				
	FFA (% of Oleic Acid)	**DAG (%)**	**DAG 32 (%)**	**DAG 34 (%)**	**DAG 36 (%)**
0	0.15 ± 0.00	11,4 ± 0.2	5.6 ± 0.3	58.4 ± 0.7	36.1 ± 0.8
14	0.16 ± 0.00	12,8 ± 0.6	5.4 ± 0.2	57.7 ± 0.6	36.9 ± 0.8
41	0.19 ± 0.00	11,3 ± 0.3	5.4 ± 0.2	58.7 ± 0.4	35.9 ± 0.3
77	0.20 ± 0.00	10,8 ± 0.6	5.7 ± 0.1	58.0 ± 0.1	36.2 ± 0.1
95	0.27 ± 0.00	11,6 ± 0.3	5.7 ± 0.1	59.0 ± 0.6	35.3 ± 0.5
140	0.26 ± 0.00	10,3 ± 0.3	5.9 ± 0.2	58.3 ± 0.3	35.8 ± 0.2
185	0.30 ± 0.00	11,7 ± 0.1	5.6 ± 0.1	58.0 ± 0.1	35.4 ± 0.6

[a] Data are presented as mean values ± standard deviation (SD). Mean values with the same letter are not different ($P \leq 0.05$).

Table 4. Oxidative parameters detected on the fresh frying oils and their changes (evolution) during frying in real restaurant conditions.

Frying Days	**Samples**	**BLEND**	
		OSI (h)	**POV (meqO2/Kg of oil)**
I	1	22,9 ± 0.2	0.8 ± 0.1
	2	17,6 ± 0.7	1.4 ± 0.2
	3	nd	5.6 ± 0.3
II	1	nd	8.1 ± 0.2
	2	24,1 ± 0.7	7.7 ± 0.3
	3	20,2 ± 0.3	4.8 ± 0.2
III	1	7,8 ± 0.5	10.3 ± 0.1
	2	6,2 ± 0.1	5.3 ± 0.2
	3	2,1 ± 0.1	16.8 ± 0.3
		PALM OIL	
I	1	19.0 ± 0.2	5.9 ± 0.2
	2	18.6 ± 0.9	5.7 ± 0.1
	3	20.2 ± 0.5	6.6 ± 0.1
II	1	19.6 ± 0.8	9.5 ± 0.8
	2	19.4 ± 0.6	5.9 ± 0.2
	3	18.2 ± 0.6	7.2 ± 0.6
III	1	18.0 ± 0.2	5.9 ± 0.3
	2	17.6 ± 0.1	4.8 ± 0.2
	3	14.3 ± 0.4	5.9 ± 0.4

[a] Data are presented as mean values ± standard deviation (SD). Mean values with the same letter are not different ($P \leq 0.05$).

Table 5 DAG content (%) and DAG distribution (%) in different classes detected on the fresh frying oils and their changes (evolution) during frying day in restaurant condition

Frying Days	Samples	BLEND				
		FFA (% Oleic Acid)	DAG (%)	DAG 32 (%)	DAG 34 (%)	DAG 36 (%)
	0	0.07 ± 0.0	4.3 ± 0.3	6.9 ± 0.4	47.1 ± 0.5	46.0 ± 0.1
I	1	0.07 ± 0.0	5.4 ± 0.5	6.2 ± 0.2	47.2 ± 0.0	46.6 ± 0.2
	2	0.07 ± 0.0	4.2 ± 0.0	6.9 ± 0.3	48.0 ± 0.3	45.2 ± 0.6
	3	0.08 ± 0.0	5.2 ± 0.1	6.4 ± 0.1	47.2 ± 0.1	46.4 ± 0.2
II	1	0.10 ± 0.0	5.4 ± 0.5	6.7 ± 0.2	48.1 ± 0.3	45.1 ± 0.0
	2	0.11 ± 0.1	4.7 ± 0.1	6.6 ± 0.2	47.4 ± 0.2	45.9 ± 0.3
	3	0.13 ± 0.0	5.1 ± 0.0	6.6 ± 0.1	47.1 ± 0.2	46.3 ± 0.4
III	1	0.14 ± 0.0	4.2 ± 0.1	7.0 ± 0.5	47.1 ± 0.6	45.9 ± 0.4
	2	0.17 ± 0.1	4.6 ± 0.1	6.8 ± 0.2	47.2 ± 0.4	46.0 ± 0.4
	3	0.18 ± 0.0	4.3 ± 0.1	6.9 ± 0.0	47.3 ± 0.2	45.9 ± 0.4
			PALM OLEIN			
	0	0.07 ± 0.0	11.6 ± 0.6	5.4 ± 0.2	58.7 ± 0.4	35.9 ± 0.3
I	1	0.07 ± 0.0	10.2 ± 0.1	5.6 ± 0.1	57.9 ± 0.2	36.5 ± 0.1
	2	0.07 ± 0.0	11.6 ± 0.1	5.4 ± 0.1	57.7 ± 0.5	36.5 ± 0.6
	3	0.07 ± 0.0	11.5 ± 0.6	5.6 ± 0.1	57.9 ± 0.1	36.9 ± 0.1
II	1	0.09 ± 0.0	11.3 ± 0.6	5.6 ± 0.1	56.0 ± 0.2	36.5 ± 0.1
	2	0.11 ± 0.0	11.0 ± 0.3	5.6 ± 0.1	57.8 ± 0.2	38.4 ± 0.1
	3	0.14 ± 0.0	11.3 ± 0.3	6.0 ± 0.4	58.1 ± 0.2	36.2 ± 0.3
III	1	0.16 ± 0.0	10.8 ± 0.9	5.8 ± 0.0	58.1 ± 0.2	36.1 ± 0.2
	2	0.19 ± 0.0	9.5 ± 0.1	5.0 ± 0.1	58.7 ± 0.1	36.3 ± 0.1
	3	0.22 ± 0.0	9.3 ± 0.1	4.6 ± 1.0	53.6 ± 0.1	41.9 ± 0.2

[a] Data are presented as mean values ± standard deviation (SD). Mean values with the same letter are not different ($P \leq 0.05$).

Table 6. Oxidative parameters detected on the fresh frying oils and their changes (evolution) during frying in real cafeteria conditions.

Frying Days	Samples	BLEND	
		OSI(h)	POV (meqO2/Kg of oil)
	0	24.6 ± 0.0	1.5 ± 0.1
I	1	22.5 ± 0.5	2.7 ± 0.1
	2	20.5 ± 0.2	4.6 ± 0.2
III	1	17.5 ± 0.6	6.7 ± 0.1
	2	17.0 ± 0.2	7.8 ± 0.6
V	1	15.4 ± 0.4	7.6 ± 0.5
VIII	1	16.0 ± 0.3	7.4 ± 0.9
	2	16.5 ± 0.1	3.5 ± 0.3
X	1	14.5 ± 0.6	6.0 ± 0.9
XII	1	18.3 ± 0.1	4.5 ± 0.4
	2	20.0 ± 0.3	7.2 ± 0.1
	PALM OIL		
	0	22.1 ± 0.0	1.5 ± 0.1
I	1	21.0 ± 0.5	4.4 ± 0.4
	2	19.4 ± 0.2	5.1 ± 0.1
III	1	19.6 ± 0.6	6.3 ± 0.2
V	1	16.8 ± 0.2	6.9 ± 0.1
VIII	1	16.3 ± 0.4	5.5 ± 0.5
	2	13.1 ± 0.3	7.0 ± 0.3
X	1	12.1 ± 0.1	3.4 ± 0.2
	2	10.4 ± 0.6	6.6 ± 0.0
XII	1	16.4 ± 0.1	6.2 ± 0.1
	2	17.9 ± 0.3	8.5 ± 0.3

[a] Data are presented as mean values ± standard deviation (SD). Mean values with the same letter are not different ($P \leq 0.05$).

Table 7 DAG content (%) and DAG distribution (%) in different classes detected on the fresh frying oils and their changes (evolution) during frying day in cafeteria condition

Frying Days	**Samples**	**BLEND**				
		FFA (% Oleic Acid)	**DAG (%)**	**DAG 32 (%)**	**DAG 34 (%)**	**DAG 36 (%)**
	0	0.07 ± 0.00	4.3 ± 0.3	6.2 ± 0.4	44.9 ± 0.2	48.9 ± 0.1
I	1	0.07 ± 0.00	4.6 ± 0.1	6.2 ± 0.0	44.9 ± 0.1	48.9 ± 0.1
	2	0.10 ± 0.00	3.8 ± 0.1	6.6 ± 0.4	46.2 ± 0.1	47.2 ± 0.4
III	1	0.11 ± 0.00	4.1 ± 0.2	6.8 ± 0.5	45.2 ± 0.4	47.9 ± 0.8
	2	0.11 ± 0.00	4.6 ± 0.1	6.5± 0.1	45.9 ± 0.4	47.6 ± 0.4
V	1	0.11 ± 0.00	4.2 ± 0.0	6.7 ± 0.0	45.9 ± 0.1	47.4 ± 0.0
VIII	1	0.12 ± 0.00	5.0 ± 0.2	7.0 ± 0.3	48.4 ± 0.0	44.6 ± 0.0
	2	0.14 ± 0.00	4.6 ± 0.3	7.2 ± 0.1	48.6 ± 0.0	44.1 ± 0.3
X	1	0.13 ± 0.00	4.7 ± 0.4	7.4 ± 0.3	48.5 ± 0.1	44.0 ± 0.0
XII	1	0.15 ± 0.00	5.5 ± 0.0	7.3 ± 0.1	48.3 ± 0.0	44.4 ± 0.3
	2	0.33 ± 0.00	7.1 ± 0.4	8.5 ± 0.1	53.6 ± 0.1	37.9 ± 0.3
		PALM OLEIN				
	0	0.15 ± 0.00	11.6 ± 0.6	5.4 ± 0.2	58.7 ± 0.4	35.9 ± 0.3
I	1	0.16 ± 0.00	11.1 ± 0.2	5.8 ± 0.1	58.5 ± 0.3	35.7 ± 0.2
	2	0.19 ± 0.01	12.4 ± 0.0	6.1 ± 0.0	58.4 ± 0.3	35.5 ± 0.3
III	1	0.20 ± 0.00	12.2 ± 0.2	6.2 ± 0.1	58.2 ± 0.2	35.6 ± 0.2
V	1	0.27 ± 0.00	10.8 ± 0.1	6.2 ± 0.0	58.5 ± 0.0	35.3 ± 0.0
VIII	1	0.26 ± 0.01	10.9 ± 0.4	6.2 ± 0.1	57.8 ± 1.0	36.1 ± 1.1
X	1	0.30 ± 0.00	10.8 ± 0.0	6.5 ± 0.1	58.3 ± 0.1	35.2 ± 0.2
	2	0.31 ± 0.00	10.1 ± 0.1	6.3 ± 0.1	58.3 ± 0.2	35.4 ± 0.3
XII	1	0.38 ± 0.01	10.8 ± 0.2	6.5 ± 0.1	58.3 ± 0.1	35.2 ± 0.2

[a] Data are presented as mean values ± standard deviation (SD). Mean values with the same letter are not different ($P \leq 0.05$).

Frying Performance of a High-Oleic Sunflower Palm Olein-blend and Palm Olein under Standarized Conditions

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Abstract

The frying performance of a vegetable oils blend (high-oleic sunflower:palm olein (55:45, v/v) was studied as a valid alternative to use of normal palm olein under frying and heated controlled condition. For the evaluation of this purpose some parameters was evaluated and monitored for 5 days of intermitting frying conditions. Thermo-oxidative, hydrolytical alterations, total polar compounds, and tocoferols and tocotrienol content were measured through various physical and chemical parameters. The oxidative stability (OSI) time reduction calculated for the frying oils was about 12% for the blend and 19% for the palm olein which was greatly related to their fatty acid (FA) composition. For the peroxide value (PV) it was observed an increase of PV with a sinusoidal trend, reaching a maximum of 8.1 meq O_2/kg of oil in the blend at the third frying day and 4.8 meq O_2/kg of oil in the palm olein at the fourth frying day. Total diacylglycerides (DAG) was stable for both blend and palm olein (6.5% vs. 9.5%), in the last case that's is related to its extraction technology. Total polar compounds were also detected by Testo 265 and by HPSEC. The fresh frying oils already presented a different total polar compounds level before being subjected to the thermal treatment (8.7% and 12.7% for the oil blend and palm olein, respectively). In both case the oils show a rapid decrease of total polar compounds during the 5 frying days of intermitting frying. During the 5-day discontinuos frying, a steady a total reduction of total polar compounds was observed in both oils and respectively 47% for the blend and 50% for the palm olein used for comparison. In general, the selected high oleic sunflower/palm oil blend (55:45, v/v) may represent a valid alternative to pure palm olein as frying medium,

Keywords: vegetable oils blend, palm olein, deep frying, oxidative parameters, hidrolytycal parameters, total polar compounds.

Introduction

Deep fried foods are becoming increasingly popular all over the world. Frying is one of the oldest methods known for food preparation. Deep-frying is a complex process that involves food immersion in an oil bath kept at high temperatures (between 160°C and 180°C), in contact with air (Gertz, 2000). The oil undergoes a large number of changes that involve a large spectrum of chemical reactions. Hydrolysis, polymerization and thermoxidation are the main reactions that occur during frying, leading to a gradual decrease of the initial oil quality and changes in the physical and chemical properties of frying bath (Gertz, 2000). The overall quality (nutritional and organoleptic) of the oil gradually fails as a result of prolonged heating, the presence of oxygen and water released from food.

The main degradation in frying oils is hydrolysis of triacylglycerols, which releases free fatty acids (FFAs), monoacylglycerols, diacylglycerols and glycerol; this phenomenon will mainly affect free acidity, total polar compounds and sensory characteristics of the frying oil. In general, the high temperatures reached during frying are among the main causes of oxidative rancidity of fats in frying baths, with the consequent generation of volatile products (responsible for unpleasant odors), modification of oil color and increase of foam and viscosity after oil polymerization; the latter, in addition, lowers the surface heat transfer coefficient, thus increasing the cooking time (Barbanti, 1993). Frying also produces volatile compounds, which are considered the most dangerous from the health stand point (Gertz, 2000) as they tend to remain in the oil bath and to be absorbed by food, thus causing an alteration of the sensory and nutritional properties of both the oil bath and the food fried therein.

Refined vegetable oils are usually employed for frying and their quality and characteristics will affect their frying performances and influence the overall quality (including flavor, texture, shelf-life and nutritional attributes)of the finished products (Dundorf, 2003) and their storage stability. The selection of a vegetable oil(s) as frying fats will not only depend on their chemical and physical characteristics, but also include on availability, economical, geographic and nutritional

issues (Blumenthal, 1996). Among the refined edible oils, palm oil is the most commonly used for industrial and domestic frying in Europe (Rossell, 1998). Its high content of saturated fatty acids (Kochhar, 2001), the low iodine value and the low levels of polyunsaturated fatty acids makes it highly resistant to thermal processes and the development of unpleasant flavor. However, while its fatty acid composition makes it very resistant to thermal processes, it also generates some great disadvantages. In fact, the high level of saturated fatty acids renders the palm oil solid at room temperature (melting point=46.5°C) (Jin et al., 2008), thus being difficult to handle it and impossible to directly pour it into the fryer. For these reasons, palm olein, the low-melting point fraction of palm oil (melting point=36 °C) (Berger, 2005) is preferred; however, the handling problems are not solved. To overcome this aspect, palm oil (or its fractions) can be mixed with other vegetable oils with a higher unsaturation degree, so as to bring down the melting point and to give rise to a more fluid blend that can satisfy the operators requirements. In addition, the characteristic high content of diacylglycerol of palm oil (7-9% vs. 2% of the other vegetable oils) (Gee et al., 2007) can favor foaming during frying as they act as surfactants, beside their contribution to the polar compound fraction generated during the frying processes.

Recently, several studies have been carried out on mixtures of edible oils that include palm oil as one of the blends' ingredients (De Marco et al., 2007; Nor Aini et al., 2005; Pangloli et al., 2002), with the purpose to solve the negative aspects caused by its presence, but without losing the thermoxidative stability that characterizes it and that is largely pursued in food frying. In general, in the present study, the selected blend high oleic sunflower/palm oil blend (55:45, v/v) may represent a valid alternative to pure palm olein as frying medium, even though this blend showed a faster increase in some oxidation indices and displayed a lower hydrolytic degradation, as confirmed by the FFA and DAG levels represent a further advantage from the technological standpoint, as it results in less foaming which allows a better handling.

The aim of this work was to evaluate the frying performance of a high-oleic sunflower oil:palm olein blend (55:45, v/v), to assess its suitability as a frying blend and as a good alternative to palm

olein. Standardized frying lab-scale conditions were used and both hydrolytic and oxidative parameters were determined

Materials and Methods

Samples

High-oleic sunflower oil and palm olein were provided by a local oil bottling company (Italy). A high-oleic sunflower oil:palm olein blend (55:45, v/v) was prepared with oils from the same production batch. Prefried potato sticks were purchased at a local store (Bologna); the potato sticks belonged to the same batch and, as declared in the label, were prefried with palm olein by the producer.

Frying experimental design

In every frying session, about 300 g of prefried potato sticks (Coop, Italia) were deep-fried for 4 min in 4.5 L of hot oil at 180°C, without replenishment. An electrical bench-top fryer RF5S (Roller Grill Italia, Italy) was used. The frying temperature was kept almost constant (±1°C), so that the potato mass to oil mass ratio (g/g) remained low (0.0667, e.g. 1:15 frying ratio). Table 1 reports the specific experimental conditions. Three intermittent frying sessions were carried out per day according to the following sequence: 3 repeated 4-min frying cycles/day, with a 60-min preheating step, a 15-min interval between frying sessions, and a 60-min end-heating step. This scheme was repeated for a total of 5 consecutive days.

Frying oils were sampled (75 mL) after 60 min and 160 min frying every day and stored at -18°C until analysis.

Reagents, solvents, and standards

Diethyl ether, double distilled water, *n*-hexane, chloroform, isopropanol, methanol, glacial acetic acid, phenolphtalein, sodium thiosulfate and starch water, were supplied by Carlo Erba Reagenti (Milano, Italy). Anhydrous sodium sulfate was purchased from BDH (BDH, England). Commercial standards of diacylglycerols (dimyristin, dipalmitin, distearin, diolein), tocopherols (α, β, γ and δ) and tocotrienols (α, γ and δ) were supplied by Sigma Aldrich (St. Louis, MO, USA). Silica solid phase-extraction (SPE) cartridges NH_2 (500 mg stationary phase/3 mL) were purchased from Phenomenex (Torrence, CA, USA). The silylation mixture was prepared with dried pyridine (ACROS Organics, USA), hexamethyldisilazane (BDH, England) and trimethylchlorosilane (Carlo Erba Reagenti, Milan, Italy) at a ratio of 5:2:1 by volume.

Determination of the total fatty acid composition

Fatty acid composition was determined by gas chromatography after derivatization to fatty acid methyl esters with KOH 2N in methanol, according to IUPAC Standard Methods 2.301 and 2.302 (IUPAC, 1992). About 50 mg of oil were dissolved in 1 mL of *n*-hexane and then transmethylated with 1 mL of 2 N KOH solution in methanol. The mixture was vigorously shaken with a vortex for 1 min and 2 µL of solution were injected into a GC equipped with a split-splittless injector and a flame ionization detector (FID). An HP-INNOWax polyethylene glycol column (30 mm length x 0.25 mm i.d. x 0.25 µm) (Agilent), was used. The oven temperature was programmed from 180°C to 230°C at 3°/min; the final temperature was kept for 5 min. The injector and detector temperatures were both set at 250°C. Hydrogen was used as carrier gas. The split ratio was 50:1.

The results were expressed as relative area percent with respect to the total FA area. One replicate was analyzed per sample.

Determination of the total tocopherol content

Tocopherols were determined by HPLC with fluorescence detection (FLD) according to the IUPAC Standard Method (Standard Method 2.507, 1992). About 50 mg of sample were added with 1 mL of *n*-hexane and 20 μL of solution was injected into the HPLC-FLD. The column was a LiChrosorb Si 60 (25.064 mm) packed with silica (5 mm particle size) (Merck, Darmstadt, Germany). Sample solutions of 50 mg/mL were used and the mobile phase was *n*-hexane:isopropanol (99:1, v/v), with a flow rate of 1 mL/min. The reading was carried out at 290 nm as $\lambda_{excitation}$ and 330 nm of $\lambda_{emission}$.

Tocopherol and tocotrienol content of the samples was expressed as ppm and one replicate was analyzed per sample.

Determination of free acidity (FA)

Free acidity value (expressed as % oleic acid) was evaluated according to the official method described in annex III of EC Regulation 2568/91 (EC Commission Regulation EEC No. 2568/91 of July 1991). Two replicates were analyzed per sample.

Gas-chromatographic determination of total diacylglycerols (DAG)

DAG were determined according to a modified version of the method suggested by Bonoli et al. (Bonoli et al., 2007), where dihydrocholesterol was used as internal standard. Seventy μL of a solution of dihydrocholesterol (1.052 mg of dihydrocholesterol in 1 mL of *n*-hexane:isopropanol (4:1, v/v)) were added to 100 mg oil and dissolved in 500 μL of *n*-hexane before loading into SPE. The rest of DAG purification by SPE elution was the same as reported by Bortolomeazzi et al. (1990). The purified fraction was then silylated (Sweeley et al., 1963), dried under nitrogen stream and dissolved in 100 μL of *n*-hexane. One microliter of the silylated solution was injected into a gas

chromatograph (GC 8000 Series Fisons Instruments, Milano, Italia) with injector spit-splittless and a flame ionization detector (FID). A fused silica capillary column (25 m x 0.25 mm i.d. x 0.1 µm of film thickness) coated with 65% diphenyl-polysiloxane–35% dimethyl-polysiloxane (TAP, Varian, Lake Forest, USA), was used. The oven temperature was programmed from 160°C to 350°C at 3°/min and kept a 350°C for 20 min. The injector and detector temperatures were set at 350°C. Helium was used as carrier gas at a flow of 1.40 mL/min (pressure, 80 kPa); the split ratio was 1:30. Total DAG content of the samples was expressed as percentage. Two replicates were analyzed per sample.

Determination of the Oxidative Stability Index (OSI)

Oxidative stability of samples were determined according to the AOCS Official Method Cd12b.92 (AOCS Official Method Cd 12b-92). The analysis were carried out by the Omnion Oxidative Stability Instrument (OSI) with eight channels (Omnion, IL). Frying oil (5.0 ± 0.1 g) was placed in a polycarbonate tube and heated at 110±1°C under atmospheric pressure and at 150 mL/min of air flow rate. This test is based on the increase in conductivity due to the formation of volatile acids (mainly formic acid) during oil accelerated oxidation (Jebe et al., 1993). The conductibility was measured in polycarbonate tubes using twice distilled water. Results were expressed as induction time (h). Two replicates were analyzed per sample.

Determination of peroxide value (PV)

Peroxide value (PV) (expressed as meq O_2/kg oil) was evaluated according to the official method described in annex III of EEC Regulation 2568/91 (EC Commission Regulation EEC No. 2568/91 of July 1991). Two replicates were analyzed per sample.

Determination of total polar compounds (TPC)

Total polar compounds were measured by Testo 265 (electronic cooking oil tester) (CP B 04/11/512 (PRO 04/11/015). The sensor based on parallel plate capacitor was immersed in the hot oil at frying temperature for about 5 min, after completion of the frying cycles. The TPC and temperature readings were carried out once the sensor had stabilized (around 1 min). The Testo 265 instrument provided a TPC value (expressed as %), with a $\pm$ 2% of standard deviation of the instrumental measurement. Two replicates were analyzed per sample.

The results obtained with the Testo measurement were compared with those obtained by gravimetric determination after fractionation in chromatographic column (Paradis, 1981), by absorption chromatography using silica mini-columns (IUPAC, 2000) and by high-performance size-exclusion chromatography (HPSEC) (Dobarganes et al., 2000). One replicate was analyzed per sample.

Determination of the smoke point

This determination was performed according to the NGD C77 method (NGD C77, 1976), by using a Cleveland instrument. The smoke point is directly correlated to the fatty acid composition of the oil; in fact, lower smoke points are generally measured in oils and fats with a high content of PUFA, due to the lower stability of the double bonds at high temperatures. The smoke point, therefore, is an important determination to assess the degradation level of an oil/fat when subjected to heating. Briefly, the oil is placed in an brass container, up to the knurling fixed level. The oil is heated and, when it starts smoking, the temperature is registered. Two replicates were analyzed per sample.

Statistical analysis

The data are reported as mean values of 2 independent replicates (*n*= 2) of each analytical determination. One-way ANOVA was performed, in order to compare data obtained for different frying times and different oils.

Results and Discussion

Fatty acid and tocopherol composition

During frying, the fatty acid (FA) composition of the edible oils can change because unsaturated FA can oxidize and thus convert into different compounds. Table 2 reports the percentage of the main FA found in the oils before (fresh) and after (160 h) the discontinuous frying process. The FA composition of the blend is correlated to the corresponding contribution of the single oils that constitute the frying oil blend. Oleic acid was the most abundant FA in both fresh frying oils (62.3% and 46.2% for the blend and palm olein, respectively), followed by palmitic (21.1% vs. 33.8%) and linolenic acids (10.9% vs. 13.2%). The total unsaturation level of the frying blend (186) was lower with respect to that of palm olein (235). Such difference can be attributed to the highest percentage of linoleic acid present in palm olein. In general, a higher amount of PUFA can impact oil's resistance to oxidation, especially when it is subjected to high temperatures (Chu & Kung, 1998). Although a high level of PUFA is not the best oil feature for a good frying performance, it is important to remember that PUFA, such linolenic and linoleic acids, are fundamental in human diet as they cannot be produced by animal metabolism (Tuberoso et al., 2007; Shahidi & Wanasundara, 1998). After frying, as expected, a decrease of PUFA and parallel increase of saturated FA (SFA) was noted in both oils. Table 3 also reports the real loss of each FA during thermoxidation, calculated as suggested by Dobarganes et al. (1988). It is possible to note that the effective FA loss was higher in the oil blend than in palm olein, especially for oleic acid.

Table 4 shows the content of tocopherols and tocotrienols of the fresh frying oils, as well as their evolution during the 5-day discontinuous frying. Tocopherols and tocotrienols are abundant in vegetable oils and are well-known for their antioxidant activity, which should protect them from thermo-oxidation (Karabulut et al., 2005; Giannazza et al., 2001; Gordon, 1990; Hoffman, 1989). Natural tocopherols and tocotrienols are retained at considerable levels in finished refined vegetable oils (Karabulut et al., 2005; Simonne et al., 1998). Since they are relatively thermal-resistant, so that only modest tocopherol losses are registered during deodorization/distillation phase (carried out at 220–260 °C) (De Greyt et al., 2000), their natural antioxidant activity should protect the refined oils against thermal oxidation. In general, the total tocopherols and tocotrienols content was more higher in the palm olein than in the blend (509 vs 462, respectively). α-tocopherol was the representative tocopherol detected in the blend; γ-tocotrienols in the palm olein. Both tocopherols and tocotrienol was subjected at decrease during frying day in both frying oils. The total content of α-tocoferol in the blend was higher than in the palm olein (249 ppm vs. 127 ppm, respectively). α-tocotrienols, δ-tocotrienols and γ-tocotrienols were more higher in the palm olein than in the blend. However, during frying, an extensive decrease of these antioxidant substances was observed in both oils. It's interesting to note like the loss in the tocopherols is more fast with respect to tocotrienol.

Oxidative and hydrolytic modifications

The oxidative stability of vegetable oils is one of the most important indicators of their quality, as lipid oxidation mainly occurs through a chain-reaction mechanism, which generates several compounds that can greatly modify their sensory and nutritional profile. According to Yavary et al. (2010), the evaluation of OSI time on crude vegetable oils (by Rancimat test or OSI instrument) cannot guarantee or predict the actual frying performance of the oil, but it can be considered a

useful "screening" test, to reduce the possibility of introducing low stability oils into the production area (Morton et al., 1988).

Table 5 shows the OSI times of the fresh oils and samples collected during the discontinuous frying steps. Significant differences were detected in the OSI times of the two frying oils. The oils actually exhibited different resistance to oxidative stress caused by frying; in fact, the OSI time reduction detected in the blend was about 12%, whereas it was about 19% in the palm olein. These data can be mainly related to the different FA compositions, since the blend had higher MUFA (62% vs. 46%) and lower PUFA percentages (11% vs. 13%); this trend is also confirmed by the corresponding unsaturation indexes (186 vs. 235 for the blend and palm olein, respectively). Besides FA composition, the contribution of natural antioxidants to the oxidative stability of oils should not be overlooked (Table 4); however, despite the palm olein displayed a higher total tocopherol content than the blend (509 vs. 462, respectively), it was not enough to further attenuate the oxidative process in this system. De Marco et al. (2007) stated that the greater intensity of the oxidative process in palm olein could be attributed to the higher content of diacylglycerols, which seem to increase the solubility of water in oil, thus promoting additional hydrolysis. FFA generated by hydrolytic process, are more prone to oxidation than esterified ones; furthermore, the free carboxylic group of the FA molecules is supposed to be responsible for its prooxidative activity (De Marco et al., 2007).

Primary oxidation products were evaluated by the determination of peroxide value (PV). Since PV is related to oxidative rancidity, its determination is essential to assess the lipid quality and, in fact, is one of the most widely used methods for testing the quality of fats and oils. However, it is not exhaustive, since peroxides are highly unstable, so they easily decompose and convert into other oxidation products. Although a linear correlation has been found between PV and the levels of odors during the initial stages of lipid oxidation (O'Brien, 1998), this method alone is not a good indicator for the oil's sensory quality. This is because PV increases up to a maximum level and then

decreases as storage time increases. In any case, a high PV indicates generally low sensory quality (present or future), but a low PV is not always an indicator of good quality.

In the present study, all fresh oils had a PV lower than the PV limit fixed for the refined oils that constituted the blend (0.07 meq O_2/kg of oil) (Stazione Sperimentale per le Industrie degli Oli e dei Grassi, 2002). In both oils, it was observed an increase of PV with a sinusoidal trend, reaching a maximum of 8.1 meq O_2/kg of oil in the blend at the third frying day (Table 5) and 4.8 meq O_2/kg of oil in the palm olein at the fourth frying day (Table 5). As reflected by such trends, peroxides are unstable under frying conditions and are formed with different velocities depending on their FA composition and unsaturation degree. In general, an increase in the peroxide value during the initial frying step would be expected to be followed by a decrease after further frying, because hydroperoxides tend to decompose at 180°C to form secondary oxidation products. The overall increase of peroxide values occurred particularly during the cooling period, where the frying oil is exposed to air at high temperature.

The smoke point of the fresh oil blend and palm olein was very similar (248°C vs. 247°C, respectively). These values are higher than those reported for most refined edible oils (180-230°C) (Matthaus, 2010). FFA and other volatile substances affect the smoke point. Although the determination of the smoke point is not an important indicator for defining the performance of frying oils, it can provide a suggestion of the maximum temperature up to which the oil can be heated. Morton and Chidley (1988) reported that the amount of smoke emanating from a cup is directly proportional to the concentration of low molecular weight decomposition products in the oil. However, the determination of smoke point through a standard procedure still heavily relies on the ability of the worker to determine the point at which the oil begins to smoke (Stevenson, 1984).

The free acidity value detected in the fresh oil blend and palm olein was very low (0.07% of oleic acid), as expected for refined oils. A gradual increase of free acidity was detected in both oils during frying day, being more intense in palm olein. These trends can be related to the higher

percentage of diacylglycerols present in the palm olein (Table 6), as they are more prone to hydrolysis as compared to triacylglycerols.

Table 6 shows the total diacylglycerol (DAG) content of the frying oils and their distribution into the three DAG characteristic groups (D_{32} (C16-C16 DAG), D_{34} (C16-C18 DAG) and D_{36} (C18-C18 DAG)). The DAG level of palm olein (9.5%) was higher than that of the oil blend (6.5%). The D_{32} was present at low percentages in these oils (5% and 6% in the blend and palm olein, respectively), if compared with the total FA composition where palmitic acid constituted 21% and 33% of total FA in the blend and palm olein, respectively. The D_{34} was the most representative DAG fraction in both frying oils (53% vs 58% in the blend and palm olein, respectively), being related to their total FA composition as it was dominated by the presence of palmitic and oleic acids. The D_{36} group was the second most abundant one, representing 42% and 36% of total DAG in the blend and palm olein, respectively. In general, significant changes in DAG content was found during the different number of frying cycles. The determination of total DAG in vegetable oils is a very important parameter, since they are not eliminated by the refining processes and, therefore, they provide a real assessment of the hydrolytic status of the vegetable oils. In addition, during frying, DAG act as surfactant molecules, leading to increase the surface tension of the frying system. The high interfacial tension in the frying system breaks steam bubbles and forms a steam blanket over the oil surface. The steam blanket reduces the contact between the oil and oxygen, and lowers the oil oxidation (Choe & Min, 2007; Blumenthal, 1991).

Table 7 reports the percentages of TPM identified in the frying oils and their distribution into the different compound classes (polymerized triacylglycerols, oxidized triacylglycerols, diacylglycerols and free fatty acids). The fresh frying oils already presented a different TPM level before being subjected to the thermal treatment (8.7% and 12.7% for the oil blend and palm olein, respectively). This difference could be attributed to the different nature of the oils as related to their chemical composition; in fact, most TPM detected are ascribable to the presence of a higher DAG percentage

in palm olein, since these compounds are not removed during the refining process. During the 5-day discontinuos frying, a steady a total reduction of TPM was observed in both oils and respectively 47% for the blend and 50% for the palm olein used for comparison (CM, 1991). Table 7 show the TPM distribution determined by HPSEC. The total increase of total polar compounds can be attributed to the increase of oxidized triacylglycerols and polymers, while the FFA and DAG content are virtually unchanged. Similar trends were noted by others authors (Dobarganes et al., 1993; Bansal et al., 2010).

TPM were also determinated by Testo 265, which provided a TPM measurement expressed as percentage. A sensor based on parallel plate capacitor was immersed in hot oil at the fixed frying temperature for about 5 min, after the completion of the designated frying cycles. The data obtained with the Testo 265 were compared with those of the official method for the TPC determination and are shown in Graphs 1 (oil blend) and 2 (palm olein). As evinced in the graphs, the TPM values obtained with Testo were significantly higher than those found with the official method, which means that Testo tends to overestimate TPM level by 5%. Since many productive realities (industry and cattering) use this easy-to-handle sensor, this overestimation should be considered, especially when subjected to controls; the Testo producer, in fact, reports a ± 2% of standard deviation of the instrumental measurement. In any case, both methods were able to confirm the observed trend in TPM determined by HPSEC.

CONCLUSIONS

The results obtained from the analytical evaluation of two frying oils led to conclude that the selected high oleic sunflower/palm oil blend (55:45, v/v) may represent a valid alternative to pure palm olein as frying medium, even though this blend showed a faster increase in some oxidation indices (OSI), mainly due to its higher unsaturation degree. However, the blend displayed a lower

hydrolytic degradation, as confirmed by the FFA and DAG levels. Moreover, the lower DAG content may represent a further advantage from the technological standpoint, as it results in less foaming which allows a better handling.

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Table 1. Experimental frying conditions

FRYING CONDITIONS	
Surface of oil exposed to air (cm^2)	850.5
Oil quantity (L)	4.5
Oil turnover rate (%)	0
Temperature (°C)	180 ± 2
Amount of potatoes fried/cycle (g)	300
Frying ratio (potato:oil in mass basis)	1:15
Frying time (min)	4
Frying frequency	intermittent
Number of frying sessions	15
Total frying time (min)	60

Table 2. Main fatty acids the frying oil blend (high-oleic sunflower oil:palm olein, 55:45, v/v) and palm olein

		C16:0	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**OTHERS**	**18:2/16:0**	**Total unsaturation***
BLEND	FRESH	21.1	0.2	3.3	62.3	10.9	0.1	2.0	0.5	186
	V DAY	23.2	0.2	3.5	61.2	9.4	0.1	2.4	0.4	
PALM OLEIN	FRESH	33.8	0.3	3.7	46.2	13.2	0.2	2.6	0.4	235
	V DAY	35.6	0.1	3.9	45.9	11.4	0.2	3.0	0.3	

[*] It was calculated by multipling the relative percentage of the various unsaturated fatty acids by different factors, depending on the oxidative stabilitty of each unsaturated fatty acid class. The factors are 1, 10 and 20 for mono-, di- and tri-unsaturated FA.

Table 3. Fatty acids loss calculated in frying oil blend (high-oleic sunflower oil:palm olein, 55:45, v/v) and palm olein

	SAMPLES	**C16:0**	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**OTHERS**
Fatty acid loss **	BLEND	34	0	4	44	11	0	3
	PALM OLEIN	21	0	3	56	9	0	2

** It was calculated as the percentage of palmitic acid (C16:0) found in the fresh oil, divided by the percentage of C16:0 in hot oil multiplied for the percentage of a fatty acid (X) in heated oil (Dobarganes et al., 1988).

Table 4 Tocopherols and tocotrienols content detected in the frying oil blend (high-oleic sunflower oil:palm olein, 55:45, v/v) and palm olein

Frying Days	Frying Time (min)	α-tocopherol	α-t3	γ-tocopherol	δ-tocopherol	γ-t3	δ-tocopherol	δ-t3	Tot tocopherol	Tot tocotrienol	Total content
		BLEND									
	0	249	76	8	0	95	0	34	257	205	462
I	160	187	54	7	0	73	0	30	193	157	351
II	160	102	28	0	0	42	0	31	102	101	203
III	160	42	12	0	0	20	0	27	42	59	101
IV	160	10	0	0	0	0	0	28	10	28	37
V	160	0	0	0	0	0	0	0	0	18	18
		PALM OLEIN									
	0	127	144	0	0	192	0	45	127	382	509
I	160	130	146	0	0	181	0	45	193	372	503
II	160	68	68	0	0	79	0	39	102	186	254
III	160	29	33	0	0	53	0	34	42	120	149
IV	160	0	0	0	0	71	0	35	10	106	106
V	160	0	0	0	0	0	0	27	0	27	27

Table 5. Some chemical parameters (oxidative stability index (OSI), peroxide value (POV), free faty acids (FFA) and smoke point) determined in the fresh frying oil blend (high-oleic sunflower oil:palm olein, 55:45, v/v) and palm olein, as well as during the 5-day discontinuous frying

		OSI (h)		POV (meq O2/kg of oil)		FFA (% oleic acid)		Smoke Point (°C)	
Frying Days	**Frying Time (min)**	**BLEND**	**PALM OLEIN**	**BLEND**	**PALM OLEIN**	**BLEND**	**PALM OLEIN**	**BLEND**	**PALM OLEIN**
	0	23.9 ± 0.0	21.6 ± 0.0	0.6 ± 0.1	2.9 ± 0.1	0.07 ± 0.00	0.07 ± 0.00	248 ± 1.4	247 ± 1.4
I	60	20.9 ± 0.0	19.0 ± 0.4	3.9 ± 0.1	3.3 ± 0.1	0.06 ± 0.01	0.08 ± 0.01	-	-
	160	19.3 ± 0.0	18.7 ± 0.0	4.8 ± 0.0	4.1 ± 0.2	0.07 ± 0.00	0.09 ± 0.02	-	-
II	60	17.3 ± 0.0	17.2 ± 0.0	5.8 ± 0.0	4.2 ± 0.1	0.08 ± 0.00	0.13 ± 0.00	-	-
	160	16.2 ± 0.2	17.2 ± 0.2	8.1 ± 0.5	3.5 ± 0.4	0.10 ± 0.00	0.15 ± 0.02	-	-
III	60	14.9 ± 0.0	15.2 ± 0.0	4.2 ± 0.2	3.7 ± 0.0	0.11 ± 0.00	0.16 ± 0.01	-	-
	160	12.4 ± 0.0	13.4 ± 0.0	3.7 ± 0.2	4.0 ± 0.0	0.14 ± 0.01	0.20 ± 0.00	-	-
IV	60	8.6 ± 0.1	10.1 ± 0.1	3.7 ± 0.0	4.7 ± 0.2	0.13 ± 0.01	0.20 ± 0.00	-	-
	160	6.4 ± 0.0	8.4 ± 0.0	4.6 ± 0.1	4.8 ± 0.1	0.17 ± 0.00	0.25 ± 0.00	-	-
V	60	3.6 ± 0.0	5.4 ± 0.0	4.7 ± 0.3	4.2 ± 0.1	0.18 ± 0.01	0.28 ± 0.00		
	160	3.0 ± 0.0	4.3 ± 0.0	4.3 ± 0.1	4.5± 0.0	0.21 ± 0.01	0.30 ± 0.01		

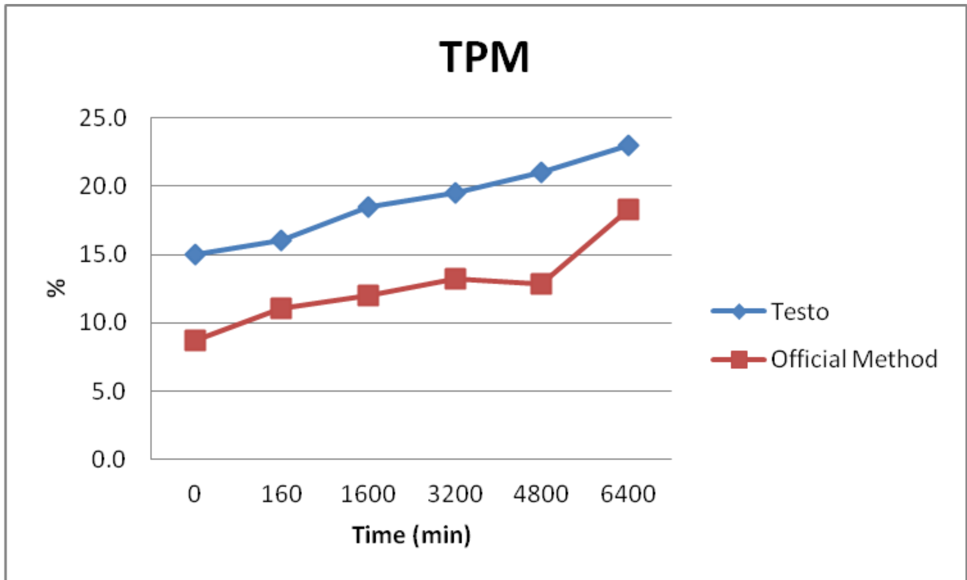
Table 6. Total DAG content (%) and distribution of the DAG classes (%) in the fresh frying oil blend (high-oleic sunflower oil:palm olein, 55:45, v/v) and palm olein, as well as during the 5-day discontinuous frying

Frying Days	Frying Time (min)	BLEND			
		DAG (%)	DAG 32 (%)	DAG 34 (%)	DAG 36 (%)
	0	6.0 ± 0.1	5.2 ± 0.1	52.8 ± 0.1	41.9 ± 0.1
I	160	6.9 ± 0.3	5.6 ± 0.3	53.5 ± 0.1	40.8 ± 0.1
II	160	4.3 ± 0.0	5.8 ± 0.3	53.6 ± 0.1	40.7 ± 0.4
III	160	4.3 ± 0.0	6.4 ± 0.4	53.9 ± 0.6	39.8 ± 1.0
IV	160	4.7 ± 0.2	6.1 ± 0.1	53.0 ± 0.1	40.9 ± 0.0
V	160	6.8 ± 0.3	6.0 ± 0.2	51.3 ± 1.2	42.7 ± 1.4
		PALM OLEIN			
	0	9.5 ± 0.1	5.0 ± 0.1	58.7 ± 0.1	36.3 ± 0.1
I	160	9.4 ± 0.2	6.1 ± 0.0	58.5 ± 0.1	35.3 ± 0.1
II	160	9.1 ± 0.3	6.6 ± 0.1	58.7 ± 0.2	34.7 ± 0.3
III	160	9.8 ± 0.0	6.6 ± 0.3	58.7 ± 0.3	34.6 ± 0.6
IV	160	7.4 ± 0.3	7.5 ± 0.3	58.7 ± 0.4	33.8 ± 0.1
V	160	7.0 ± 0.2	7.4 ± 0.1	58.5 ± 0.2	33.8 ± 0.3

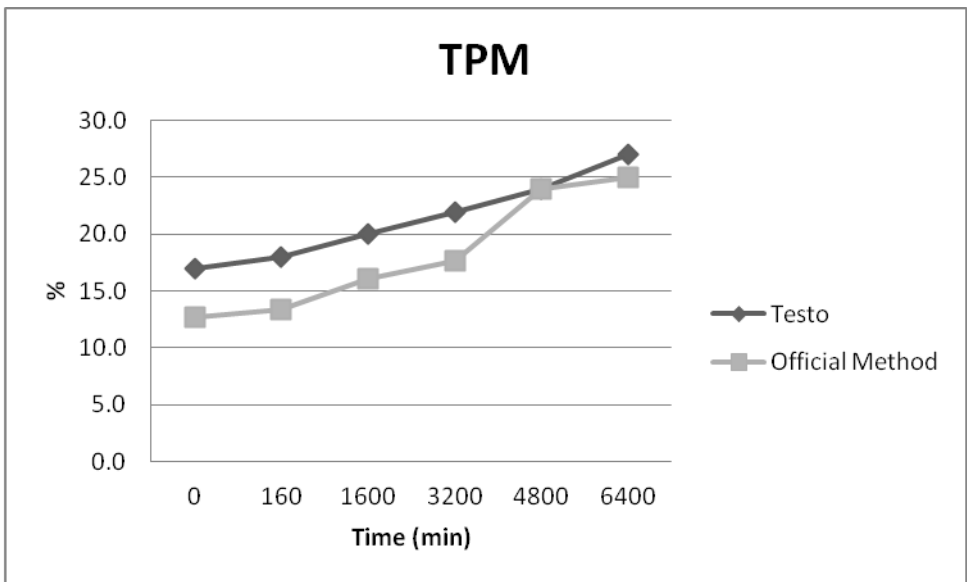
Table 7. TPM and distribution of polar compounds determined by HPSEC in the fresh frying oil blend (high-oleic sunflower oil:palm olein, 55:45, v/v) and palm olein, as well as during the 5-day discontinuous frying

Frying Days	**Frying Time (min)**	**BLEND**					
		TPM (%)	**POL (%)**	**DIMERS (%)**	**OTAG (%)**	**DAG (%)**	**FFA (%)**
	0	8.7	0.0	0.0	1.9	6.8	0.0
I	160	11.1	0.0	1.1	2.5	7.3	0.2
II	160	12.0	0.3	2.4	3.5	5.8	0.0
III	160	13.2	0.5	3.1	4.1	5.4	0.0
IV	160	12.8	0.6	3.3	4.1	4.8	0.0
V	160	18.3	1.1	5.4	5.8	5.9	0.0
		PALM OLEIN					
	0	12.7	0.0	0.0	1.0	11.7	0.0
I	160	13.4	0.0	1.0	1.7	10.7	0.0
II	160	16.1	0.0	2.8	3.5	9.8	0.0
III	160	17.7	0.0	4.0	3.8	9.9	0.0
IV	160	23.9	0.9	5.3	5.5	12.3	0.0
V	160	25.1	1.3	6.3	5.6	11.8	0.0

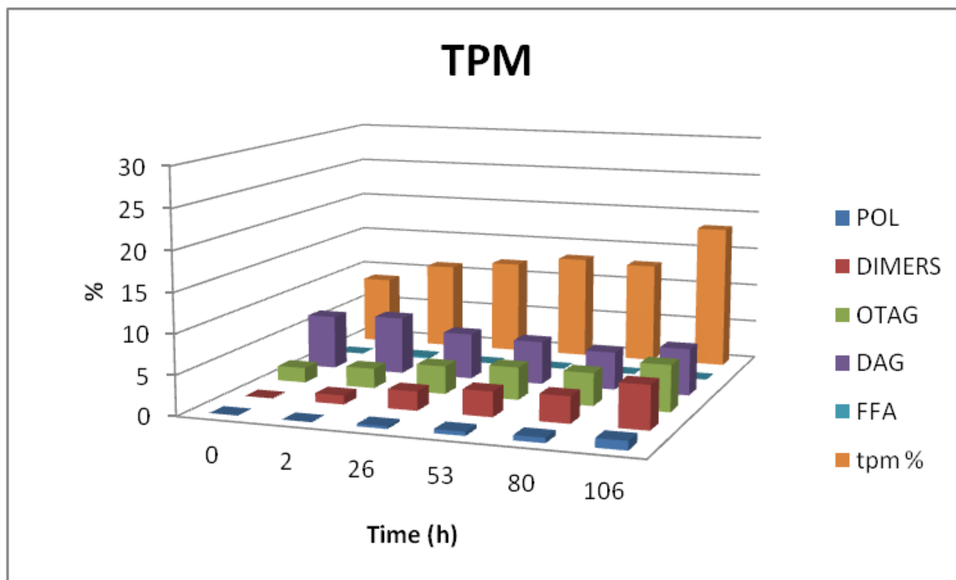
Abbreviations: TPM, total polar material; POL, polymerized triacylglycerols; OTAG, oxidized tryacylglycerols; DAG, diacylglycerols; FFA free fatty acids.



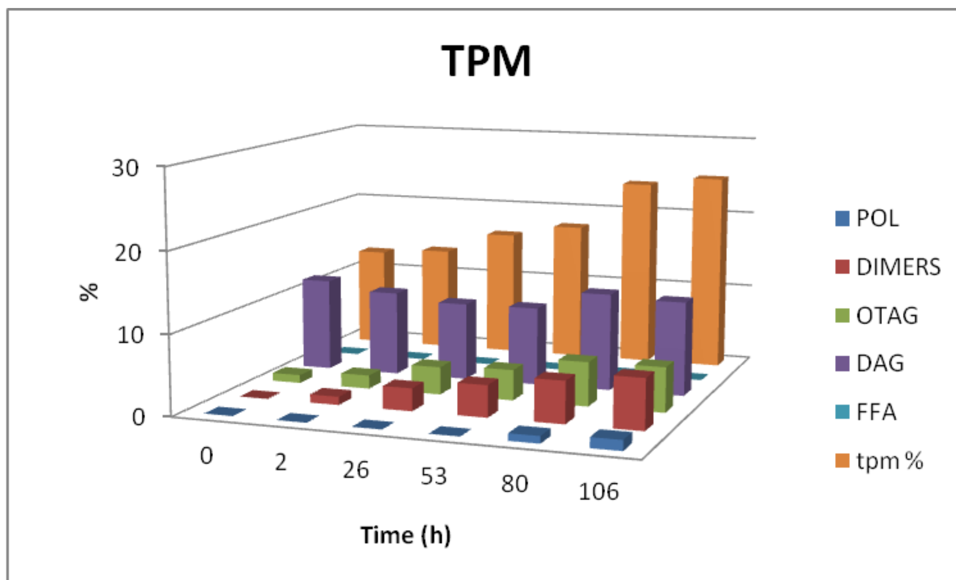
Graph 1. Evolution of TPM in the frying oil blend (high-oleic sunflower oil:palm olein, 55:45, v/v), determined by Testo 265 and the Official method .



Graph 2. . Evolution of TPM in palm olein, determined by Testo 265 and the Official method .



Graph 3. Evolution of TPM and the PC classes in the frying oil blend (high-oleic sunflower oil:palm olein, 55:45, v/v), detected by HPSEC



Graph 4. Evolution of TPM and the PC classes in palm olein, detected by HPSEC

Chemical Performance of Extravirgin Olive Oil as Compared with Vegetable Oils, During Repeated Deep-Frying

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Abstract

The aim of this work was to evaluate the frying performance of an EVOO (cv. Coratina) under intermittent frying and to compare it with a frying oil blend (high oleic sunflower oil:palm oil, 60:40, v/v), characterized by a high percentage of MUFA. Standardized frying lab-scale conditions were used and both hydrolytic and oxidative parameters were determined. For the evaluation of this purpose some parameters was evaluated and monitored for 5 days of intermitting frying conditions. Thermo-oxidative, hydrolytical alterations, total polar compounds, and tocoferols and tocotrienol content were measured through various physical and chemical parameters. The oxidative stability (OSI) time reduction calculated for the frying oils was about 12% for the EVOO and 10% for the blend which was greatly related to their fatty acid (FA) composition. For the peroxide value (PV) it was observed an increase of PV with a sinusoidal trend, reaching a maximum of 7.5 meq O_2/kg of oil in the blend and around 4.8 meq O_2/kg of oil in the extra virgin olive oil. Total diacylglycerides (DAG) was stable for both extravirgin olive oil and blend (1.5% vs. 4.3%), in the last case that's is related to its extraction technology. Total polar compounds were also detected by Testo 265 and by HPSEC. The fresh frying oils already presented a different total polar compounds level before being subjected to the thermal treatment (11.0% and 12.0% for the extra virgin olive oil and oil blend, respectively). In both case the oils show a rapid decrease of total polar compounds during the 5 frying days of intermitting frying. During the 5-day discontinuos frying, a steady a total increase of total polar compounds was observed in both oils. In general, the extra virgin olive oils isn't a good oils for frying used in this experimental frying conditions.

Keywords: extravirgin olive oil, vegetable oils blend, deep frying, oxidative parameters, hidrolytycal parameters, total polar compounds.

Introduction

Consumption of deep-fried food has strongly increase in the last decades, especially since the consumption of frozen food and fast food has become more and more important (Matthaus, 2006). The economic impact of the frying industry amounts to about $ 83 billion of U.S. dollars and almost double for the rest of the world (Pedreschi et al., 2005). The frying process implies food immersion in an oil bath kept at high temperatures (between 160°C and 180°C), in contact with air (Gertz, 2000). This type of food processing operation causes a series of physico-chemical modifications in both oil and food, which depend on the nature of the oil, the type of food, the amount of food to be fried and the processing conditions (temperature, time, holding conditions, oil exchange). During frying, oils/fats are involved in a series of chemical changes, such a as hydrolysis, thermal degradation, oxidation and polymerization. When the edible oil has a high content of unsaturated fatty acids (FA), such degradation process can lead to great modications of their physical and chemical properties.

Several oils and fats can be utilized for frying, which can be of animal (lard) or vegetable origin (such as olive oil, palm oil, sunflower oil). The choice of the frying oil depends on many factors, such as availability, price, taste, nutritional quality and stability (Brinkmann, 2000). One of the main properties that is seeked in a frying oil, is a high resistance to oxidation during prolonged exposure to high temperatures. The choice is often influenced by local habits, which is the case of the Mediterranean area, where extravirgin olive oil (EVOO) is the most commonly used oil due its abundance and that is widely used is the use of extravirgin olive oil for seasoning in both stages of frying due to its abundance. EVOO is a valuable oil, which is obtained only by physical extraction means and it is not subjected to refining (Olias et al., 1993) Its typical chemical composition and nutritional quality is superior as compared to those of the other vegetable oils. EVOO is extracted from olive fruits (*Olea Europeae L.*) and its fragrant and delicate flavors has made it increasingly appreciated worldwide. The distinctive aroma of virgin olive oil is attributed to a large number of chemical compounds of different chemical classes, such as aldehydes, alcohols, esters,

hydrocarbons, ketones, furanes and probably others compound not unidentified (Boccouri et al., 2008; Kalua et al., 2007; Vichi et al., 2003; Kiritsakis et al., 1998). One of the most important nutritional aspects of EVOO is related to its high content of biophenols and tocopherols, which are well-known for their antioxidant activity as they help contrasting the free radical action. Moreover, it has been proven that the consumption of EVOO helps preventing many chronical diseases, especially cardiovascular diseases ones (Beauchamp et al., 2005) and recently it has also an anti-tumoral action has also been reported (Hamdi et al., 2005). Its elevated content of natural antioxidants, together with its high level of monounsaturated fatty acids (MUFA), makes EVOO particularly stable from the oxidative standpoint, and thus quite interesting for frying purposes. Its high resistance to oxidation is further supported by its low content of linoleic (C18:2) and linolenic acids (C18:3). As a consequence of its FA composition, EVOO is also characterized by a low melting point, which can facilitate oil handling during frying and provide good frying performances; this enables that the oil can be easily removed from fried food by absorption on a paper as it does not solidifies at room temperature.

The aim of this work was to evaluate the frying performance of an EVOO (cv. Coratina) under intermittent frying and to compare it with a frying oil blend (high oleic sunflower oil:palm oil, 60:40, v/v), characterized by a high percentage of MUFA. Standardized frying lab-scale conditions were used and both hydrolytic and oxidative parameters were determined.

Materials and Methods

Samples

A monovarietal EVOO (cv. Coratina) from a southern Italian region (Apulian), was used. The oil was obtained from handpicked olives in 2010 and produced using a discontinuous cold extraction systems. EVOO was stored in 5-L stainless steel containers without headspace at room temperature until their utilization.

High-oleic sunflower oil and palm olein were provided by a local oil bottling company (Italy). A high-oleic sunflower oil:palm olein blend (60:40, v/v) was prepared with oils from the same production batch. Prefried potato sticks were purchased at a local store (Bologna); the potato sticks belonged to the same batch and, as declared in the label, were prefried with palm olein by the producer.

Frying experimental design

In every frying session, about 300 g of prefried potato sticks (Coop, Italia) were deep-fried for 4 min in 4.5 L of hot oil at 180°C, without replenishment. An electrical bench-top fryer RF5S (Roller Grill Italia, Italy) was used. The frying temperature was kept almost constant (±1°C), so that the potato mass to oil mass ratio (g/g) remained low (0.0667, e.g. 1:15 frying ratio). Table 1 reports the specific experimental conditions. Three intermittent frying sessions were carried out per day according to the following sequence: 3 repeated 4-min frying cycles/day, with a 60-min preheating step, a 15-min interval between frying sessions, and a 60-min end-heating step. This scheme was repeated for a total of 5 consecutive days.

Frying oils were sampled (75 mL) after 60 min and 160 min frying every day and stored at -18°C until analysis.

Reagents, solvents, and standards

Diethyl ether, double distilled water, *n*-hexane, chloroform, isopropanol, methanol, glacial acetic acid, phenolphtalein, sodium thiosulfate and starch water, were supplied by Carlo Erba Reagenti (Milano, Italy). Anhydrous sodium sulfate was purchased from BDH (BDH, England). Commercial standards of diacylglycerols (dimyristin, dipalmitin, distearin, diolein), tocopherols (α, β, γ and δ) and tocotrienols (α, γ and δ) were supplied by Sigma Aldrich (St. Louis, MO, USA). Silica solid phase-extraction (SPE) cartridges NH_2 (500 mg stationary phase/3 mL) were purchased from Phenomenex (Torrence, CA, USA). The silylation mixture was prepared with dried pyridine (ACROS Organics, USA), hexamethyldisilazane (BDH, England) and trimethylchlorosilane (Carlo Erba Reagenti, Milan, Italy) at a ratio of 5:2:1 by volume.

Determination of the total fatty acid composition

Fatty acid composition was determined by gas chromatography after derivatization to fatty acid methyl esters with KOH 2N in methanol, according to IUPAC Standard Methods 2.301 and 2.302 (IUPAC, 1992). About 50 mg of oil were dissolved in 1 mL of *n*-hexane and then transmethylated with 1 mL of 2 N KOH solution in methanol. The mixture was vigorously shaken with a vortex for 1 min and 2 µL of solution were injected into a GC equipped with a split-splittless injector and a flame ionization detector (FID). An HP-INNOWax polyethylene glycol column (30 mm length x 0.25 mm i.d. x 0.25 µm) (Agilent, US), was used. The oven temperature was programmed from 180°C to 230°C at 3°/min; the final temperature was kept for 5 min. The injector and detector temperatures were both set at 250°C. Hydrogen was used as carrier gas. The split ratio was 50:1.

The results were expressed as relative area percent with respect to the total FA area. One replicate was analyzed per sample.

Determination of the total tocopherol content

Tocopherols were determined by HPLC with fluorescence detection (FLD) according to the IUPAC Standard Method (Standard Method 2.507, 1992). About 50 mg of sample were added with 1 mL of *n*-hexane and 20 μL of solution was injected into the HPLC-FLD. The column was a LiChrosorb Si 60 (25.064 mm) packed with silica (5 mm particle size) (Merck, Darmstadt, Germany). Sample solutions of 50 mg/mL were used and the mobile phase was *n*-hexane:isopropanol (99:1, v/v), with a flow rate of 1 mL/min. The reading was carried out at 290 nm as $\lambda_{excitation}$ and 330 nm of $\lambda_{emission}$.

Tocopherol and tocotrienol content of the samples was expressed as ppm and one replicate was analyzed per sample.

Determination of free acidity (FA)

Free acidity value (expressed as % oleic acid) was evaluated according to the official method described in annex III of EC Regulation 2568/91 (EC Commission Regulation EEC No. 2568/91 of July 1991). Two replicates were analyzed per sample.

Gas-chromatographic determination of total diacylglycerols (DAG)

DAG were determined according to a modified version of the method suggested by Bonoli et al. (Bonoli et al., 2007), where dihydrocholesterol was used as internal standard. Seventy μL of a solution of dihydrocholesterol (1.052 mg of dihydrocholesterol in 1 mL of *n*-hexane:isopropanol (4:1, v/v)) were added to 100 mg oil and dissolved in 500 μL of *n*-hexane before loading into SPE. The rest of DAG purification by SPE elution was the same as reported by Bortolomeazzi et al. (1990). The purified fraction was then silylated (Sweeley et al., 1963), dried under nitrogen stream and dissolved in 100 μL of *n*-hexane. One microliter of the silylated solution was injected into a gas

chromatograph (GC 8000 Series Fisons Instruments, Milano, Italia) with injector spit-splittless and a flame ionization detector (FID). A fused silica capillary column (25 m x 0.25 mm i.d. x 0.1 µm of film thickness) coated with 65% diphenyl-polysiloxane–35% dimethyl-polysiloxane (TAP, Varian, Lake Forest, USA), was used. The oven temperature was programmed from 160°C to 350°C at 3°/min and kept a 350°C for 20 min. The injector and detector temperatures were set at 350°C. Helium was used as carrier gas at a flow of 1.40 mL/min (pressure, 80 kPa); the split ratio was 1:30. Total DAG content of the samples was expressed as percentage. Two replicates were analyzed per sample.

Determination of the Oxidative Stability Index (OSI)

Oxidative stability of samples were determined according to the AOCS Official Method Cd12b.92 (AOCS Official Method Cd 12b-92). The analysis were carried out by the Omnion Oxidative Stability Instrument (OSI) with eight channels (Omnion, IL). Frying oil (5.0 ± 0.1 g) was placed in a polycarbonate tube and heated at 110±1°C under atmospheric pressure and at 150 mL/min of air flow rate. This test is based on the increase in conductivity due to the formation of volatile acids (mainly formic acid) during oil accelerated oxidation (Jebe et al., 1993). The conductibility was measured in polycarbonate tubes using twice distilled water. Results were expressed as induction time (h). Two replicates were analyzed per sample.

Determination of peroxide value (PV)

Peroxide value (PV) (expressed as meq O_2/kg oil) was evaluated according to the official method described in annex III of EEC Regulation 2568/91 (EC Commission Regulation EEC No. 2568/91 of July 1991). Two replicates were analyzed per sample.

Determination of total polar compounds (TPC)

Total polar compounds were measured by Testo 265 (electronic cooking oil tester) (CP B 04/11/512 (PRO 04/11/015). The sensor based on parallel plate capacitor was immersed in the hot oil at frying temperature for about 5 min, after completion of the frying cycles. The TPC and temperature readings were carried out once the sensor had stabilized (around 1 min). The Testo 265 instrument provided a TPC value (expressed as %), with a ± 2% of standard deviation of the instrumental measurement. Two replicates were analyzed per sample.

The results obtained with the Testo measurement were compared with those obtained by gravimetric determination after fractionation in chromatographic column (Paradis, 1981), by absorption chromatography using silica mini-columns (IUPAC, 2000) and by high-performance size-exclusion chromatography (HPSEC) (Dobarganes et al., 2000). One replicate was analyzed per sample.

Determination of the smoke point

This determination was performed according to the NGD C77 method (NGD C77, 1976), by using a Cleveland instrument. The smoke point is directly correlated to the fatty acid composition of the oil; in fact, lower smoke points are generally measured in oils and fats with a high content of PUFA, due to the lower stability of the double bonds at high temperatures. The smoke point, therefore, is an important determination to assess the degradation level of an oil/fat when subjected to heating. Briefly, the oil is placed in an brass container, up to the knurling fixed level. The oil is heated and, when it starts smoking, the temperature is registered. Two replicates were analyzed per sample.

Statistical analysis

The data are reported as mean values of 2 independent replicates (n= 2) of each analytical determination. One-way ANOVA was performed, in order to compare data obtained for different frying times and different oils.

Results and Discussion

Fatty acid and tocopherol compositions

Table 2 reports the fatty acid composition of the EVOO and high oleic sunflower:palm oil blend, before frying and after 5-day discontinuous frying . The FA composition of the blend is correlated to the corresponding contribution of the single oils that constitute the frying oil blend.

Oleic acid was the most abundant FA in both fresh frying oils (78% and 69% for EVOO and the blend, respectively), followed by palmitic (10.7% vs. 16.6%) and linoleic acids (7.1% vs. 9.5%). Nevertheless, the total unsaturation level of EVOO (183) was lower than that of the blend (222). Such difference can be attributed to the highest percentage of linoleic acid present in the blend. In general, a higher amount of polyunsaturated FA (PUFA) can impact oil's resistance to oxidation, especially when it is subjected to high temperatures (Chu & Kung, 1998). Although a high level of PUFA is not the best oil feature for a good frying performance, it is important to remember that PUFA, such linolenic and linoleic acids, are fundamental in human diet as they cannot be produced by animal metabolism (Tuberoso et al., 2007; Shahidi & Wanasundara, 1998).

After frying, as expected, a decrease of PUFA and parallel increase of saturated FA (SFA) was noted in both oils. Table 3 also reports the real loss of each FA during thermoxidation, calculated as suggested by Dobarganes et al. (1988). It is possible to note that the effective FA loss was higher in EVOO than in the blend, especially for oleic acid.

Table 4 shows the content of tocopherols and tocotrienols of the fresh frying oils, as well as their evolution during the 5-day discontinuous frying. Tocopherols and tocotrienols are abundant in vegetable oils and are well-known for their antioxidant activity, which should protect them from thermo-oxidation (Karabulut et al., 2005; Giannazza et al., 2001; Gordon, 1990; Hoffman, 1989). Natural tocopherols and tocotrienols are retained at considerable levels in finished refined vegetable oils (Karabulut et al., 2005; Simonne et al., 1998) and they act as radical scavengers, thus stopping the autoxidation chain reaction.

The total tocopherol content was higher in the blend (354 ppm) than in the EVOO sample (144 ppm), being α-tocopherol the most abundant one in both oils (342 ppm and 135 ppm, respectively). Tocotrienols were only detected in the blend, whose presence could be mainly ascribed to palm olein; α- and γ-tocotrienols were the most representative ones. Similar values of what were found in the literature, even though the levels may vary depending on the seed oil variety and genetic factors (Chu et al., 1998; Grela et al., 1995; Kurilich et al.,1999; Velasco et al., 2002). The antioxidant activity of EVOO is principally attributed to biophenols compounds, which provide the characteristic bitter taste and pungency (Provellini et al., 1997; Dionisi et al., 1995; Perrini, 1992). However, during frying, an extensive decrease of these antioxidant substances was observed in both oils.

Oxidative and hydrolytic modifications

The stability of vegetable oils depends on various parameters, especially on the FA composition, the content of natural antioxidants, the presence of oxygen and the storage conditions. The FA composition of oils is well-known to affect the quality and stability of oils, as the degree of FA unsaturation will greatly impact the oxidation velocity (Frankel, 1980). The oxidative stability of vegetable oils is one of the most important indicators of their quality, since several lipid oxidation products can greatly modify their sensory and nutritional profile. According to Yavary et al. (2010), the evaluation of OSI time on crude vegetable oils (by Rancimat test or OSI instrument) cannot guarantee or predict the actual frying performance of the oil, but it can be considered a useful "screening" test, to reduce the possibility of introducing low stability oils into the production area (Morton et al., 1988).

Table 5 shows the OSI times of the fresh oils and samples collected during the discontinuous frying steps. Significant differences were detected in the OSI times of the two frying oils. The fresh EVOO had a very high OSI time with respect to that of the blend (39.4 vs. 25.4, respectively). The high

OSI value of EVOO might be related to the presence of phenol that characterize oils produced with the Coratina cultivar (Chiavaro et al., 2010). The high oxidative stability of EVOO is mainly due to its FA composition, in particular to the MUFA to PUFA ratio, as well as to the presence of biophenols that also have a major role in preventing oxidation. Phenolic compounds can inhibit oxidation by a variety of mechanisms based on radical scavenging, hydrogen atom transfer, and metal-chelating attributes (Roginsky et al., 2005; Huang et al., 2005; Decker et al., 2005).

During frying, both vegetable oils exhibited similar resistance to the oxidative stress caused by the thermal process; in fact, the OSI time reduction in EVOO detected was about 12% after the-5 day frying , whereas it was about 10% for the blend. In this case, probably, the different content in substances with antioxidant activities specially in EVOO is not notable because the phenolic compounds are thermolable and in this frying condition that cannot complete their function (Rajalakshmi et al., 1997).

Primary oxidation products were evaluated by the determination of peroxide value (PV). Since PV is related to oxidative rancidity, its determination is essential to assess the lipid quality and, in fact, is one of the most widely used methods for testing the quality of fats and oils. However, it is not exhaustive, since peroxides are highly unstable, so they easily decompose and convert into other oxidation products. Although a linear correlation has been found between PV and the levels of odors during the initial stages of lipid oxidation (O'Brien, 1998), this method alone is not a good indicator for the oil's sensory quality. This is because PV increases up to a maximum level and then decreases as storage time increases. In any case, a high PV indicates generally low sensory quality (present or future), but a low PV is not always an indicator of good quality.

In the present study, the PV value detected in the fresh EVOO was 3.76 meq O_2/kg, which is in agreement with the limit fixed by the Reg. CEE 2568/91 (≤20 meq O_2/kg) , whereas the level of PV of the frying blend (1.20 meq O_2/kg) was slightly higher thant the limit fixed for the refined oils that constituted the blend (0.07 meq O2/kg of oil) (Stazione Sperimentale per le Industrie degli Oli e dei

Grassi, 2002). During frying, EVOO demonstrated to be more stable under the frying conditons tested, as the formation of peroxides in EVOO was much slower than in the blend; in the latter, an increase of PV with a sinusoidal trend was observed, reaching a maximum of 7.5 meq O_2/kg of oil at the 5th frying day (Table 5). As reflected by such trends, peroxides are unstable under frying conditions and are formed with different velocities depending on their FA composition, their unsaturation degree and the quali-quantitavive antioxidant composition. In general, an increase in the peroxide value during the initial frying step would be expected to be followed by a decrease after further frying, because hydroperoxides tend to decompose at 180°C to form secondary oxidation products.

The smoke point of the fresh EVOO and the oil blend was very similar (248°C vs. 254°C, respectively). These values are higher than those reported for most refined edible oils (180-230°C) (Matthaus, 2010); the presence of FFA and other volatile substances may affect the smoke point. Although the determination of the smoke point is not an important indicator for defining the performance of frying oils, it can provide an idea aboutthe maximum temperature up to which the oil can be heated. Morton and Chidley (1988) reported that the amount of smoke emanating from a cup is directly proportional to the concentration of low molecular weight decomposition products in the oil. However, the determination of smoke point through a standard procedure still heavily relies on the ability of the worker to determine the point at which the oil begins to smoke (Stevenson, 1984). The free acidity value detected in the fresh EVOO was in agreement with the limit fixed by the Reg. CEE 2568/91 (≤0.8% of oleic acid) , whereas and in the blend the free acidity was lower than the limit fixed for the refined oils that constituted the blend (0.07% of oleic acid). A gradual increase of free acidity was detected in both oils during frying day (≤ 0.30% of Oleic Acid).

Table 6 shows the total diacylglycerol (DAG) content of the frying oils and their distribution into the three DAG characteristic groups (D_{32} (C16-C16 DAG), D_{34} (C16-C18 DAG) and D_{36} (C18-C18 DAG)). The DAG level of EVOO (1.5%) was lower as compared to that of the blend (4.3%). The

low level of total DAG detected in EVOO suggests that it was obtained from good quality olive drupes. DAG were much higher in the blend, due to the presence of palm olein, which is obtained through an extraction technology that favors oil hydrolysis and thus DAG release and accumulation. The D_{32} group was present at low percentages in both frying oils (3% and 6% in EVOO and the blend, respectively), if compared with the total FA composition where palmitic acid constituted 11% and 17% of total FA in EVOO and the blend, respectively. The D_{34} was the most representative DAG fraction in the blend (46.3% vs. 17%), being related to its total FA composition as it was dominated by the presence of palmitic and oleic acids. The D_{36} group was the second most abundant one, representing 47% and 85% of total DAG in the blend and EVOO, respectively. In general, no significant changes were observed in the DAG content of both oils during the different frying cycles; in fact, the final DAG level was equal to 2.2% and 4.6% in EVOO and the blend, respectively. The determination of total DAG in vegetable oils is a very important parameter, since they are not eliminated by the refining processes and, therefore, they provide a real assessment of the hydrolytic status of the vegetable oils. In addition, during frying, DAG act as surfactant molecules, leading to an increase of the surface tension of the frying system. The high interfacial tension in the frying system breaks steam bubbles and forms a steam blanket over the oil surface. The steam blanket reduces the contact between the oil and oxygen, and lowers the oil oxidation (Choe & Min, 2007; Blumenthal, 1991).

Table 7 reports the percentages of TPM found in the frying oils and their distribution into the different compound classes (polymerized triacylglycerols, oxidized triacylglycerols, diacylglycerols and free fatty acids). The fresh frying oils already presented a different TPM level before being subjected to the thermal treatment (3.7% and 9.2% for the EVOO and the blend, respectively). This difference could be attributed to the different nature of the oils as related to their chemical composition; in fact, most TPM detected in the blend are ascribable to the high DAG percentage present in palm olein, since these compounds are not removed during the refining process (Gee,

2007). Since DAG constitute, together with oxidized triglycerides and polymer of triacylglycerides, the largest fraction of polar compounds, a fresh frying oil with a higher DAG initial content will reach faster the maximum level recommended for polar compounds. During the 5-day discontinuos frying, the TPM increased from 3.7% to 12.4% in EVOO and from 9.2% to 17.4% in the blend. In both cases, the percentage of TPM detected in the last frying day were below the limit suggested by the Italian Regulation (25%) (CM, 1991). Table 7 shows the TPM distribution determined by HPSEC. The total increase of total polar compounds can be attributed to the increase of oxidized triacylglycerols and polymers, while the FFA and DAG content remained virtually unchanged; this is actually confirmed by the free acidity value and the total DAG content. Similar trends were noted by others authors (Dobarganes et al., 1993; Bansal et al., 2010). TPM were also determined by Testo 265, which provided a TPM measurement expressed as percentage. A sensor based on parallel plate capacitor was immersed in hot oil at the fixed frying temperature for about 5 min, after the completion of the designated frying cycles.

The data obtained with the Testo 265 were compared with those of the official method for the TPC determination and are shown in Graphs 1 (EVOO) and 2 (oil blend). As evinced in the graphs, the TPM values obtained with Testo were significantly higher than those found with the official method, which means that Testo tends to overestimate TPM level by 5%. Since many productive realities (industry and catering) use this easy-to-handle sensor, this overestimation should be considered, especially when subjected to control; the Testo producer, in fact, reports a $\pm$ 2% of standard deviation of the instrumental measurement. In any case, both methods were able to confirm the observed trend in TPM determined by HPSEC.

Conclusions

The frying performance of an EVOO (cv. Coratina) under intermittent frying and to compare it with a frying oil blend (high oleic sunflower oil:palm oil, 60:40, v/v), characterized by a high percentage of MUFA in Standardized frying lab-scale conditions were used and both hydrolytic and oxidative parameters were determined. In this experimental situation, the extravirgin olive oil did not show a good resistance to oxidative stress. This is probably due to the fact that the antioxidant component in it present, when used in a frying condition were impaired by high temperatures. The extra virgin olive oils is the best oil for crude use and not for frying intermittent condition.

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Table 1. Experimental frying conditions

FRYING CONDITIONS	
Surface of oil exposed to air (cm^2)	850.5
Oil quantity (L)	4.5
Oil turnover rate (%)	0
Temperature (°C)	180 ± 2
Amount of potatoes fried/cycle (g)	300
Frying ratio (potato:oil in mass basis)	1:15
Frying time (min)	4
Frying frequency	intermittent
Number of frying sessions	15
Total frying time (min)	60

Table 2. Main fatty acids the frying oil extra virgin olive oil (EVOO) and blend (high-oleic sunflower oil:palm olein, 60:40, v/v)

		C16:0	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**OTHERS**	**18:216:0**	**Total unsaturation***
EVOO	FRESH	10.7	0.5	2.1	77.9	7.1	0.7	1.0	0.7	
	V DAY	12.7	0.5	2.3	76.2	6.4	0.5	1.5	0.5	183
BLEND	FRESH	16.6	0.2	3.0	68.7	9.5	0.1	1.9	0.6	
	V DAY	18.6	0.2	3.2	67.5	8.2	0.1	2.3	0.4	222

* It was calculated by multipling the relative percentage of the various unsaturated fatty acids by different factors, depending on the oxidative stabilitty of each unsaturated fatty acid class. The factors are 1, 10 and 20 for mono-, di- and tri-unsaturated FA.

Table 3. Fatty acids loss calculated in the frying oil extra virgin olive oil (EVOO) and blend (high-oleic sunflower oil:palm olein, 60:40, v/v)

	SAMPLES	**C16:0**	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**OTHERS**
**Fatty acid loss ** **	EVOO	11	0	2	64	5	0	1
	BLEND	17	0	3	60	7	0	2

Table 4 Tocopherols and tocotrienols content detected in the frying oil extra virgin olive oil (EVOO) and blend (high-oleic sunflower oil:palm olein, 60:40, v/v)

Frying Days	**Frying Time (min)**	**EVOO**									
		α-tocopherol	α-t3	β-tocopherol	δ-tocopherol	γ-t3	δ-tocopherol	δ-t3	Tot tocopherol	Tot tocotrienol	Total content
	0	135	-	-	9	-	-	-	144	-	144
I	160	129	-	-	7	-	-	-	146	-	146
II	160	77	-	-	0	-	-	-	77	-	77
III	160	35	-	-	0	-	-	-	35	-	35
IV	160	9	-	-	0	-	-	-	9	-	9
V	160	2	-	-	0	-	-	-	2	-	2
		BLEND									
	0	342	67	12	0	77	0	29	354	173	528
I	160	307	55	12	0	73	0	30	319	157	476
II	160	138	23	9	0	32	0	27	146	82	228
III	160	49	2	0	0	16	0	25	49	43	92
IV	160	2	2	0	0	0	0	0	2	2	4
V	160	0	0	0	0	0	0	0	0	0	0

Table 5. Some chemical parameters (oxidative stability index (OSI), peroxide value (POV), free fatty acids (FFA) and smoke point) determined in the fresh frying extra virgin olive oil (EVOO) and blend (high-oleic sunflower oil:palm olein, 60:40, v/v), as well as during the 5-day discontinuous frying

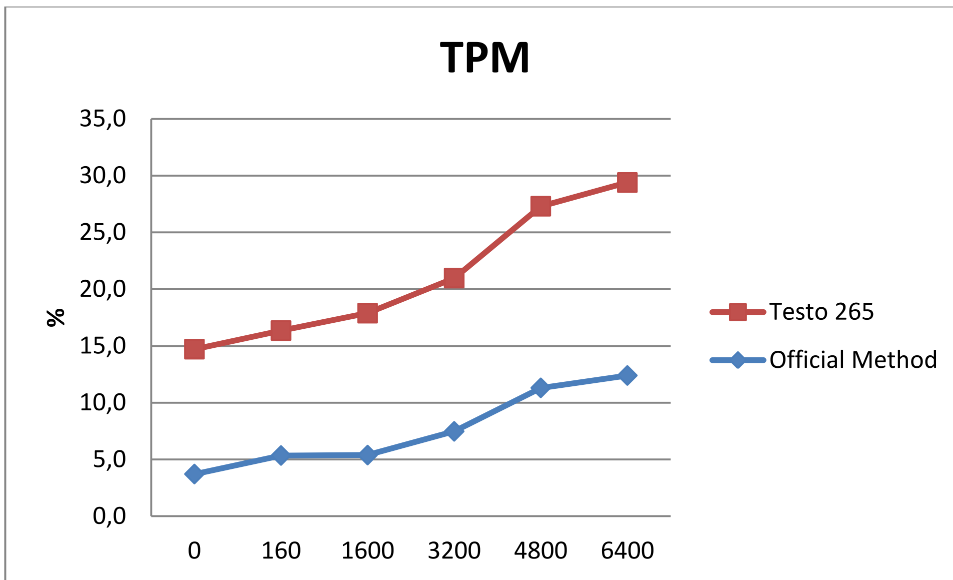
		OSI (h)		POV (meq O2/kg of oil)		FFA (% Oleic Acid)		Smoke Point (°C)	
Frying Days	**Frying Time (min)**	**EVOO**	**BLEND**	**EVOO**	**BLEND**	**EVOO**	**BLEND**	**EVOO**	**BLEND**
	0	39.4 ± 0.3	25.4 ± 0.4	3.8 ± 0.1	1.2 ± 0.0	0.20 ± 0.00	0.07 ± 0.00	247 ± 1.4	254 ± 1.4
I	60	37.1 ± 0.7	21.5 ± 0.1	3.6 ± 0.5	5.5 ± 0.0	0.19 ± 0.00	0.09 ± 0.00		
	160	34.4± 0.2	19.7 ± 0.1	4.1 ± 0.5	7.5 ± 0.0	0.18 ± 0.00	0.07 ± 0.00		
II	60	30.6 ± 0.1	18.1 ± 0.1	4.0 ± 0.2	5.1 ± 0.0	0.18 ± 0.00	0.08 ± 0.00		
	160	27.3 ± 0.6	16.3 ± 0.1	4.1 ± 0.4	6.9 ± 0.0	0.20 ± 0.02	0.11 ± 0.00		
III	60	23.6 ± 1.1	14.2 ± 0.2	4.2 ± 0.2	4.4 ± 0.0	0.21 ± 0.00	0.11 ± 0.00		
	160	18.5 ± 0.2	11.5 ± 0.5	4.1 ± 0.8	4.2 ± 0.0	0.23± 0.00	0.13 ± 0.00		
IV	60	13.3 ± 0.2	7.8 ± 0.0	4.1 ± 0.2	5.2 ± 0.0	0.24 ± 0.00	0.16 ± 0.00		
	160	9.5 ± 0.2	5.4 ± 0.0	4.0 ± 0.2	6.3 ± 0.1	0.26 ± 0.00	0.18 ± 0.00		
V	60	5.7 ± 0.1	3.1 ± 0.0	4.3 ± 0.4	7.5 ± 0.2	0.26 ± 0.00	0.21 ± 0.00		
	160	5.0 ± 0.1	2.7 ± 0.0	4.3 ± 0.3	5.6 ± 0.1	0.28 ± 0.00	0.22 ± 0.00		

Table 6. Total DAG content (%) and distribution of the DAG classes (%) in the fresh frying extra virgin olive oil (EVOO) and in the blend (high-oleic sunflower oil:palm olein, 60:40, v/v) and palm olein, as well as during the 5-day discontinuous frying

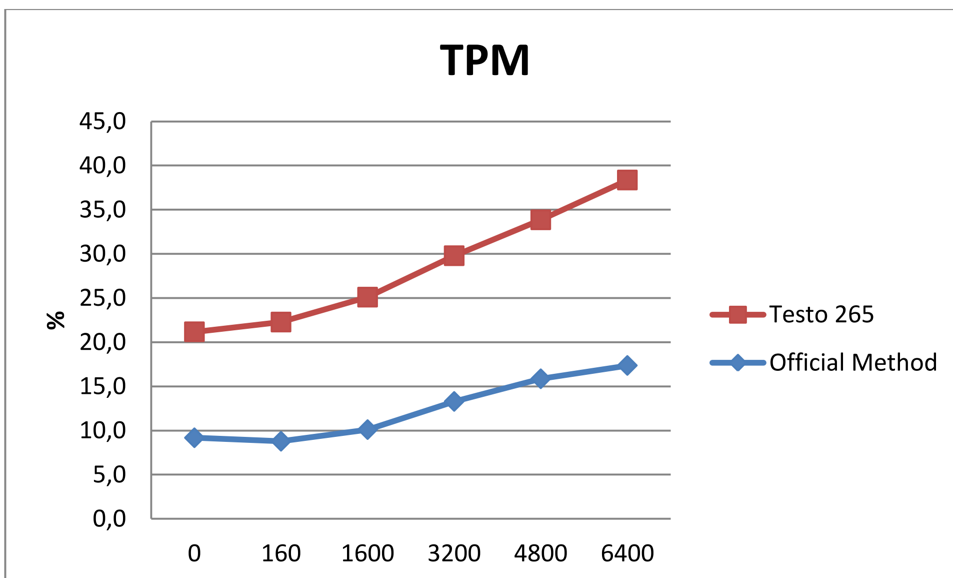
Frying Days	**Frying Time (min)**	**EVOO**			
		DAG (%)	DAG 32 (%)	DAG 34 (%)	DAG 36 (%)
	0	1.5 ± 0.1	nd	14.2 ± 0.9	85.8 ± 0.9
I	160	1.8 ± 0.6	2.2 ± 0.2	13.8 ± 5.4	83.1 ± 7.7
II	160	1.5 ± 0.1	2.3 ± 0.6	13.8 ± 5.4	83.1 ± 7.7
III	160	2.0 ± 0.1	2.7 ± 0.4	20.5 ± 0.5	76.7 ± 0.6
IV	160	2.2 ± 0.2	3.1 ± 0.3	20.9 ± 0.3	76.0 ± 0.6
V	160	2.2 ± 0.2	4.0 ± 0.4	22.1 ± 1.1	73.9 ± 0.6
		BLEND			
	0	4.3 ± 0.3	6.9 ± 0.4	47.1 ± 0.5	46.0 ± 0.4
I	160	4.3 ± 0.6	5.7 ± 0.1	46.9 ± 0.2	47.4 ± 0.1
II	160	4.6 ± 0.8	5.8 ± 0.2	46.9 ± 0.5	47.4 ± 0.7
III	160	3.8± 0.2	6.5 ± 0.3	45.1 ± 0.7	48.4 ± 0.4
IV	160	4.3 ± 0.6	6.5 ± 0.4	46.0 ± 0.7	47.5 ± 0.2
V	160	4.6 ± 0.6	6.2 ± 0.3	46.5 ± 0.5	47.2 ± 0.3

Table 7. TPM and distribution of polar compounds determined by HPSEC in the fresh frying extra virgin olive oil (EVOO) and in the blend (high-oleic sunflower oil:palm olein, 60:40, v/v) and palm olein, as well as during the 5-day discontinuous frying

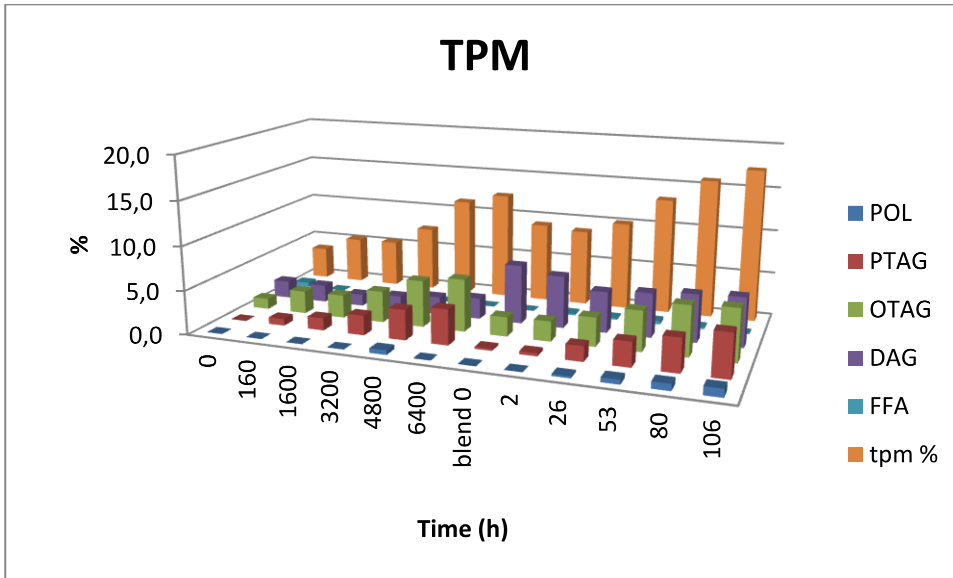
Frying Days	**Frying Time (min)**	**EVOO**					
		TPM (%)	POL (%)	DIMERS (%)	OTAG (%)	DAG (%)	FFA (%)
	0	3.7	0.0	0.0	1.2	2.0	0.0
I	160	5.3	0.0	0.8	2.6	2.0	0.0
II	160	5.4	0.0	1.4	2.6	1.3	0.0
III	160	7.5	0.0	2.3	3.6	1.6	0.0
IV	160	11.3	0.5	3.4	5.3	2.0	0.0
V	160	12.4	0.0	4.0	6.0	2.4	0.0
		BLEND					
	0	9.2	0.0	0.2	2.2	6.7	0.0
I	160	8.8	0.0	0.3	2.3	6.0	0.2
II	160	10.1	0.2	1.7	1.7	4.7	0.2
III	160	13.3	0.5	2.8	2.8	5.1	0.4
IV	160	15.9	0.7	3.8	3.8	5.4	0.2
V	160	17.4	0.9	4.9	4.9	5.6	0.0



Graph 1. Evolution of TPM in the EVOO determined by Testo 265 and the Official method .



Graph 2. Evolution of TPM in the frying oil blend (high-oleic sunflower oil:palm olein, 60:40, v/v), determined by Testo 265 and the Official method .



Graph 3. Evolution of TPM and the PC classes in the EVOO and in frying oil blend (high-oleic sunflower oil:palm olein, 60:40, v/v), detected by HPSEC

Inhibitory Effect of Liposomal Solution of Grape Seed Extract on the Formation of Heterocyclic Amines

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Abstract

Nutrition plays a vital role in maintaining good health. There have been several studies which highlight the role of cause and effect between diet and disease, particularly if harmful substances are presence in foods. Heterocyclic aromatic amines (HAA), which are formed in meat and fish cooked at high temperatures (≤ 150° C) have shown to exhibit carcinogenic and mutagenic activities. HAA are formed when creatine, creatinine, sugar and amino acids are heated together. Their formation appears to be dependent on time, frying temperature, and the food composition. Several studies have demonstrated the progressive decrease of HAA in the presence of compounds with antioxidant activity such as vitamine E, or polyphenolic compounds. Our studies have shown that the activity of such antioxidants may be maintained and/or enhanced by encapsulation. One potential system for the delivery of polphenolic compounds is liposomes; vesicles composed of a lipid bilayer. Our previous studies have shown that liposomes may be manufactured to contain high concentrations of e.g. grape seed extract due to a high affinity of such compounds for the phospholipid bilayer. The aim of this work was to assess the effectiveness of liposomal-encapsulated grape seed extract instead of the typical oil marinade to inhibit the production of HAA during the frying of beef patties.

Keywords: Liposome, grape seed extract, heterocyclic amines, pan-frying, antioxidant activity

Introduction

Nutrition plays a vital role to maintain a good human health, so several studies have been carried out to highlight the role of cause and effect between diet and disease, especially if harmful substances are present in food. Heterocyclic aromatic amines (HAA), which are formed in meat and fish when cooked at high temperatures (≥150°C) have shown to exhibit carcinogenic and mutagenic activities. This formation mainly depends on temperature and thus HAA are classified at least in 2 groups due to the formation process. HAAs formed at temperatures between 100 and 300°C, are known as "thermic HAAs," IQ type, or aminoimidazoazarenes and the others formed at higher temperatures, above 300°C, are known as "pyrolytic HAAs," or non-IQ type (Alaejos et al., 2011). Their concentration seems to depend on meat type, cooking, parameters (duration, temperature, equipment and methods), pH, water activity, creatine concentration, amount and type of carbohydrates and free amino acids (Oz et al., 2011). Several studies have demonstrated the progressive decrease of HAA in the presence of compounds with antioxidant activity, such as vitamin E, fruit extracts, plant extract carotenoids, spices and biophenols (Gibis et al., 2010, Smith et al., 2008, Murkovic et al., 1998); in particular, the addition of the latter can have positive effects on the reduction of toxic compounds generated by cooking processes. Biophenols are widely-spread natural antioxidants, which help preventing lipid oxidation and, thus, they play multiple positive roles on human health, including anticarcinogenic activity (Bendini et al., 2007). These potent antioxidants, however, can loose their activity when subjected to high temperatures or exposed to oxygen or light sources.

In recent years, much emphasis has been placed on the production of functional food, nutraceutical compounds and enriched food, with the aim to improve and compensate the nutrition shortages resulting from lifestyle or generated by cooking methods and/or technological processes. In order to ensure their integrity and release, the compounds with antioxidant activity can be encapsulated in several systems that can warrant their protection from the environment, as well as their

bioavailability. The utilization of encapsulated polyphenols instead of free compounds can overcome their unstability drawbacks, alleviate unpleasant tastes or flavors, and improve their bioavailability and *in vivo* and *in vitro* half-life (Fang et al., 2010). Different studies have demonstrated that the biophenols activity may be maintained and/or enhanced by encapsulation (Mc Clements, 2010). Encapsulation allows the inclusion of substances within another materials or system, and it has been succesfully applied in several industrial sectors (pharmaceutical, cosmetics, chemicals and food). Encapsulation can be performed by different techniques, such as spray drying, spray chilling, extrusion coating, fluidized bed coating, coacervation, inclusion complexation, centrifugal extrusion, rotational suspension separation and liposome entrapment (Gibbis, 1999). The latter is widely utilized as carriers of bioactive compounds (Mozafari, 2008) and they mainly consist in a lipid bilayer that enclose an aqueous compartment, which allows to convey molecules with different properties (hydrophilic, lipophilic and amphipatic) and characteristics, thus maintaining the stability and functionality of the encapsulated substances. Encapsulation, therefore, is a powerful technological tool that can be extremely helpful to produce high-quality food, with a controlled delivery of bioactive compounds, which can lead to an enhanced absorption of such molecules and to a more stable food system with an extended shelf-life (Chaundrhy, 2008). Moreover, such properties can be further exploited to remove undesirable molecules (such as HAA) from thermally-treated food, to improve its overall quality and nutritional profile.

The aim of this work was to evaluate the efficiency of encapsulation of biophenols from grape seed extracts in liposomal systems and to assess their actual impact on HAA formation in cooked beef patties, previously marinaded with the grape seed liposome solution. This application arises from the need to decrease the formation of toxic and mutagenic compounds (such as HAA) that are generated during the cooking at high temperatures, so as to improve the nutritional profile of such complex food products and to reduce the harmful effects on human health.

Materials and Methods

Materials

The soy lecithin (Lipoid S75, Lipoid) was bought to Ludwigshafen, Germany; Grape seed extract was provided by the nature network, Martin Bauer Group, Plantextrakt GmbH & Co.KG. (Vestenbergsgreuth, Germany). Sephadex Gel, methanol, acetonitrile per HPLC, Triethylamine, ortophosphoric acid 20%. Extraction phase was carried out with Silica Phase Extraction (SPE), PRS (500 mg), C18 (500 mg and 100 mg), Varian (Canada, USA).

The HAA-standards IQ (2-amino-3-methylimidazo[4,5-*f*] quinoline), IQx (2-amino-3-methylimidazo[4,5-*f*] quinoxaline), MeIQ (2-amino-3,8-dimethylimidazo [4,5-*f*] quinoline), MeIQx (2-amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline), 4,8-DiMeIQx (2-amino-3,4,8 trimethylimidazo[4,5-*f*] quinoxaline), 7,8-DiMeIQx (2-amino-3,7,8 trimethylimidazo[4,5-*f*] quinoxaline), PhIP (2-amino-1- methyl-6-phenylimidazo[4,5-*b*] pyridine), Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido [4,3-*b*] indole), Trp-P-2 (3-amino-1-methyl-5H-pyrido [4,3-*b*] indole), Glu-P-1 (2-amino-6- methyldipyrido[1,2-*a:* 3',2'-*d*] imidazole), Glu-P-2 (2-Aminodipyrido [1,2-*a:* 3',2'-*d*] imidazole), AαC (2-amino-9H- pyrido[3,4-*f*] indole), norharmane (9H-pyrido[3,4-*b*] indole) and Harman (1-methyl-9H-pyrido[3,4-*b*] indole) with the following end concentration in the standard mixture 19.7, 22.8, 21.3, 13.6, 12.9, 12.5, 6.5, 5.9, 7.4, 9.4, 5.3, and 5.0 ng in 100 μL of methanol, were obtained from Toronto Research Chemicals, (Ontario, Canada). All stock solution of each substance were corrected by means of extinction coefficient (Hatch, Felton, Stuermer, & Bjeldanes, 1984). Caffeine (internal standard 2.5 μg/mL in ultrapure water and methanol, 1+1, v/v).

Preparation of liposomes

Liposomes were prepared with different percentage of soy lecithin (1%, 2% and 5%, respectively). The soy lecithin (Lipoid S75, Lipoid, Ludwigshafen, Germany) was dissolved in an acetate buffer system containing two concentrations of grape seed extract (0.1 and 0.2%, respectively). The

mixture was passed 5 times through a high pressure homogenizer at 22500 psi (Microfluics M-110 EH, Newton, USA) to form fine disperse liposomes.

Liposomal charge and size measurements

Liposomal charge and liposomal particles diameter was determined by dynamic light scattering (Zetasaizer Nano Z-S, model ZEN3600, Malvern instrument, Worchester, UK) at 25°C. The samples were diluted approximately 9 time with the buffer and transfer in the cuvettes for size determination (DTS1060, Malvern Instruments). The particle size and zeta potential were determined right after emulsions preparation and for the four consecutive weeks. Each measurement was repeated twice time at 25°C.

Determination of Total Phenolics Compounds by Folin Ciocolteau Assay

Grape seed extract was provided by the nature network, Martin Bauer Group, Plantextrakt GmbH & Co.KG. (Vestenbergsgreuth, Germany) at a total amount of polyphenols ≥ 40%, calculated as gallic acid. Two extract solutions (0.1% and 0.2%) were prepared by dissolving the dried grape seed extract in acetate buffer (pH 3.8 ± 0.2; 0.25 M) under stirring for 30 minutes followed by filtering using folded filters. The amount of phenolic compounds in the extract, in liposomes after encapsulation and after gel filtration was determined with the Folin-Ciocalteu assay (Singleton, et al 1999).

Liposomes were deliberately destabilized by adding 3 mL of 0.15 wt% Triton X100 to samples for the determination of phenolics in bilayers.

The photometric measurements was carried out at 720 nm allowing reagents to react for 60 min. The results were expressed as reference gallic acid (gallic acid mg/g extract).

Sampling and Pan-frying trials

Deep frozen beef patties were obtained from Salomon Hitburger (Großostheim, Germany). A total of 72 deep frozen beef patties were marinated with 10 g of marinade solution (n=6 for each experimental line); a total of 3 control lines (control, marinated with rapeseed oil; grape seed extract, marinated with pure extract 0.1 and 0.2%, respectively) and 3 liposome lines (liposome 1%, 2% and 5% of soy lecithin), were investigated. For each liposome line, two extract solutions were encapsulated (0.1% and 0.2%). The marinated beef patties were fried on a double contact grill (Nevada, Neumarker, Hemer, Germany), heated at 220°C for 2:40 min as recommended by Gibis et al. (2010).

The meat samples were weight before and after cooking step for the individuation of percentage loss of weight. Average cooking losses of around 34-40%.

The meat samples were stored under vacuum conditions in the frozen.

Determination of Weight Loss and Color

For the determination of weight loss, the patties (n=6) were weighted before and 1 h after frying. The L*a*b*-values of the fried patties were determined using a Chroma Meter CR 200 (Minolta, Osaka, Japan) 1 hour after heating. Six patties of every batch were each measured 3 times.

The color's determination was effectuated on each patties after cooking in three times by Chroma Meter CR 200 (Minota, Osaka, Japan).

Extraction and determination of HAA

Polar and non polar HAA were analyzed using a modified HPLC method (Gibis et al., 2007) based on a method described by Gross and Grueter (1992). A 30 g of samples was homogenised to Ultraturrax with 9 0g of NaOH (1 M/L). The mixture was divided in four parts of 20 g; two parts was spiked with Internal Standard Mixture (100 µL). The different aliquotes was homogenized with diatomaceous materials (18 g) and fill in Extrelut columns. The Propyhilsulphonic acid silica

column (PRS) was put in tandem (or on line) whit extrolut column. A fine needles was putted in the end of cartridges column for flow reduction and the HAA compounds were extracted with a mixuter of dichloromthane:toluene (95:5, v/v) (80 mL). The extraction was stopped at 40 mL of dichloromethane:toluene (95:5, v/v). The Extrelut was discarded and the PRS was dried on the vacuum. The PRS cartridges column was cleaning with 6 mL of hydrochloric acid (HCl) 0.01 M/L. For the eluition of apolar portion, 15 mL of methanol-hydrochloric acid (0.1 M/L) and 2 mL of ultrapure water was used. The solvent was collected in a empty extrelut column. A 500 µL of ammonium acetate and 25 mL of ultrapure water were fill in the column. A C18 SPE (500 mg) activated with 2 mL of methanol and 2 mL of ultrapure water were put on-line whit the column. The solvent was eluted in the SPE column on the positive vacuum. A 2 mL of water was rinsed and the cartridges was dried and positive vacuum. The apolar amine was eluted in the vial using 1200 µL of methanol-ammonium solution (9:1, v/v). A C18 SPE (100 mg) activated with 1 mL of methanol and 1 mL of ammonium acetate (pH 8) were put on-line whit the PRS column. A 20 mL of ammonium acetate and 2 mL of ultrapure water was eluted. The polar amine was eluted in the vials using 800 µL of methanol-ammonium solution (9:1, v/v). Apolar and polar fraction were concentrated under nitrogen and ridissolved in 100 µL of Coffein Acid (IS).

Peaks were identified by comparing retention times and spectra with standards.

The HPLC equipment consisted of a Gynkotek HPLC-system (Gynkotek, Germering, Germany) Pump M480, autosempler Gina 50, degasser (DG 1310 S), with a fluorescence detector (RF 1200), diode array detector (UVD 320) and Gynkosft chromatography data system (version 5.50). The HAA were analyzed using column, TSK-gel ODS-80TM 250-4.6 mm, 5 µm (Tosoh Bioscence, Stuttgart, Germany) and a guard column Supelguard ^{TM} LC-18-DB (Supelco, USA). The mobile phase consisted of eluent A: 0.01 M Triethylamine phosphate buffer (pH 3.2), eluent B: 0.01 M Triethylamine phosphate buffer (pH 3.6), eluent C: acetonitrile. HAA were separated with a gradient program at 1 mL/min 82-75% A, 10% B and 8-15% from 0 to 10 min; 85-75% B and 15-25% C from 10 to 20 min, 0-5% A, 75-55% B and 25-45% C from 20 to 29 min; 5-82% A, 55-10%

B and 45-8% C from 29 to 33 min. For the regeneration of HPLC column, the mobile phase was 75% C for 4 min and then equilibrated for 10 min with the starting conditions to 52 min.

UV-detection was at 258 nm and 3D-field for spectral plots (200-360 nm). The adjustment of fluorescence detector (ex/em) was from 0 min (360/450 nm), 14 min (300/440 nm), 22 min (265/410 nm), 24 min (305/390 nm), 25.5 min (265/410 nm) and 28 min (335/410 nm).

Results and discussion

Determination of liposomal stability

For each liposomal preparation with and without extract, the diameter was measured immediately and monitored for four consecutive weeks. The differences in particle diameter between the control (without extract) and liposomes with different extract concentrations and different percentage of soy lechitine already suggest that the polyphenolic compounds had been incorporated in the liposomal bilayer since liposomes with extract were larger than liposomes without, and respectively (3, 2.5 and 1.6-fold, for each experimental trials). During the four weeks of storage particle diameters remained virtually unchanged, a sign of the high stability of the liposomal preparation. The Figure 1.1 a showed that the diameters in the liposomes without extract is significant smaller than the liposome with grape seed extract. The more smaller particle size is showed in the liposome 5% trial where the diameter is the most smaller in comparison with the trials 1% and 2%. The analysis of ζ-potential it's possible to understand the efficiency of antioxidant compound to explain their stability on the charge of liposomes.

Determination of Total Phenolics Compounds by Folin Ciocolteau Assay

Analysis of the polyphenolic content showed that most of the polyphenolic compounds had been incorporated in the bilayer membrane rather than the interior of the liposomes. This amount in the bilayers was affected by the lecithin concentration used. The Figure 1 show the linear regression and it is the results of particle diameter versus the loadings of grapeseed extract. The loading was calculated like a percentage ratio between the grape seed extract and soy lecithin. It's possible to see that the higher loadings caused larger diameters of liposome solution.

Determination of Weight Loss and Color

The weight loss during frying was determined as the differences of the weight of the deep frozen beef patties before and after frying step. Internal temperature was monitored continually in the treatment and it's was registered on average around 72°C.

Effect on formation of HAA in patties.

The second part of this study was focused on to identify the effectiveness of release of compounds with antioxidant activity from liposomal encapsulated system. Two most important heterocyclic aromatic amine like MeIQx and PhPI for the polar fraction was identified, and Norharman and Harman β-carbolines for the apolar fraction. The recovery found in this samples are showed in Figures 1.2 and suggest the higher efficiency of the method used. The average of recoveries for the HAA were 61% for MeIQx, 47% for PhIP, 55% for Noharman and Harman and that are comparable with other studies. In Figure 2 it's possible to see the HPLC chromatogram detected for the polar fraction of spiked and unspiked samples. The content of HAA in fried beef patties is showed in Table.1 The most predominant HAA found in the meat samples for the polar fractions was MeIQx. In general, the analysis of concentrations of HAA formed in beef patties apparently showed the positive effect of the presence of encapsulated polyphenolic compounds. It's possible to note the

significant decrease of MeIQx; that was observed in control samples (marinated with rapeseed oil) in comparison with all samples and not significant difference was found in different experimental line marinated with liposome encapsulation. It's interesting to note, that significant difference was detected in the control trial, in particular between the samples marinated with rapeseed oil and the samples marinated with pure grape seed extract, and in this last case, no distinction was found between the two different percentage of extract solution (0.1% and 0.2%, respectively). Significant difference was detected between the control line (marinated with rapeseed extract) and the encapsulation trials (1%, 2% and 5% of soy lecithine) with an increase of 1.6-fold, without distinction between a different percentage of soy lecithin. Different studies have shown the positive effect exerted by molecules with anitioxidant activity in the inhibition of formation of HAA in general with most particular focus on MeIQx. In this experiment, the antioxidant effect is exerted in presence of molecules with antioxidant activity and that are not necessarily encapsulated. No significant difference was found between the patties marinated with pure grape seed extract in comparison with the liposomal encapsulated trials. This trend is due to the mechanism of formation of the polar amine that are generated by the reduction of hexose and amino acids via Maillard reactions and Strecker degradation reaction to go trought a free radical intermediate from of the pyridines and pyrazines to reacting with creatine (Tsen et. al, 2006; Milic et al., 1993). The antioxidant action exerted by phenolic compounds of grape seed extract is carried out with no significant differences between encapsulated and not encapsulated, with a consequent lack of protection function. In this contest, it's interesting to note that for each experimental line, no difference was found between the control line of liposome (liposome without grape seed extract) and encapsulation trial. The apparent positive impact on the diminution of this polar compounds is not attributable to the action performed only by phenolic compounds but can be probably attributed to synergistic antioxidant effect exerted by soy lecithin The determination of PhIP shows a different pattern in comparison with the other polar HAA determinated. In this case, significant differences are found between the control and samples treated with grape seed extract with no distinction

between the two concentration of polyphenolic compounds. In this case, the optimum antioxidant effect from the polyphenolic compounds is dispatch. Significant differences are detected in the trials with liposomes 1% of soy lecithyne. In this case, the differences are showed between liposome without grapeseed extract and liposome with grape seed extract without distinction of concentration of this. No significant differences are detected for the trial liposome 2% and liposome 5%.

The β-carboline Norharman and Harman was detected in quantity range from 8.92 to 11.87 ng/g for Norharman and from 3.3 and 5.7 ng/g for Harman. This data are comparable with the other study (Totsuka at. Al., 1999). In this case is possible detected no effect from the antioxidant compounds without distinction beetwen the samples marinated with pure grapeseed extract and the samples marinated with the encapsulated extract with different percentage of spy lecithine in the formulation of liposomes and different concentration of antioxidant compounds. This data is motivated by the mechanism of formation of Norhman and Harman that is not involved a free radical action and for this reason can not be subject to the action exerted by antioxidant phenolic molecules (Tsen et al., 2006). In the pathway of formation of this compounds, triptophane was identified as precursor and glucose is enhancing Norharman formation. In an aqueous model systems containing creatine, glucose and varius single amino acids, Noharman and Harman were formed in mixtures containing isoleucine, arginine, phenylalanine and tryptphane, while tyrosione afforded norharman upon heating at 180 °C for 10 min. When iron (Fe^{2+}) and copper (Cu^{2+}) were added to a model system Nohorman was formed even at temperatures as low as 40°C (Pfau et al., 2004).

Abbreviations Used

HAA: Heterocyclic Aromatic Amines, IQ: 2-Amino-3-methylimidazo[4,5-*f*]quinoline (CAS No.: 76180-96-6), IQx: 2-Amino-3-methylimidazo[4,5-*f*]quinoxaline (CAS No.: 108354-47-8), MeIQ: 2-Amino-3,4-dimethylimidazo [4,5-*f*]quinoline (CAS No.: 77094-11-2), MeIQx: 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (CAS No.: 77500-04-0), 4,8-DiMeIQx: 2-Amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (CAS No.: 95896-78-9), 7,8-DiMeIQx: 2-Amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (CAS No.: 92180-79-5), PhIP: 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (CAS No.: 105650-23-5), Trp-P-1: 3-Amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (CAS No.: 62450-06-0), -monoacetate (CAS No.: 68808-54-8), Trp-P-2: 3-Amino-1-methyl-5H-pyrido[4,3-*b*]indole (CAS No.: 62450-07-1), Glu-P-1: 2-Amino-6-methyldipyrido[1,2-a:3',2'-*d*]imidazole (CAS No.: 67730-11-4), Glu-P-2: 2-Aminodipyrido [1,2-*a*:3',2'-*d*]imidazole (CAS No.: 67730-10-3), AαC: 2-Amino-9H-pyrido[2,3-*b*]indole (CAS No.: 26148-68-5), MeAαC: 2-Amino-3-methyl-9H-pyrido[2,3-*b*]indole (CAS No.: 68006-83-7), Harman: 1-Methyl-9H-pyrido[3,4-*b*]indole (CAS No.: 486-84-0), Norharman: 9H-pyrido[3,4-*b*]indole (CAS No.: 244-63-3).

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Table 1: Content of HAA in fried beef patties

	Control (rapeseed oil)	Control I (pure grapeseed extract 0.1%)	Control II (pure grapeseed extract 0.2%)	Liposome 1% of soy lecithine	Lip 1% 0.1% Grapeseed Extract	Lip 1% 0.2% Grapeseed Extract
MeIQx	1.93 ± 0.06	1.16 ± 0.07	1.28 ± 0.27	1.24 ± 0.09	0.99 ± 0.13	1.05 ± 0.10
PhIP	1.00 ± 0.21	0.49 ± 0.12	0.75 ± 0.21	1.19 ± 0.04	0.51 ± 0.15	0.90 ± 0.11
Norharman	9.23 ± 0.60	8.92 ± 0.60	11.29 ± 0.27	9.43 ± 0.05	9.15 ± 0.64	9.02 ± 0.45
Harman	3.8 ± 0.26	3.3 ± 0.11	4.0 ± 0.20	4.6 ± 0.02	4.7 ± 0.24	4.6 ± 0.78
	Liposome 2% of soy lecithine	Lip 2% 0.1% Grapeseed Extract	Lip 2% 0.2% Grapeseed Extract	Liposome 5% of soy lecithine	Lip 5% 0.1% Grapeseed Extract	Lip 5% 0.2% Grapeseed Extract
MeIQx	1.03 ± 0.05	0.92 ± 0.18	1.30 ± 0.01	1.38 ± 0.14	1.17 ± 0.17	1.36 ± 0.39
PhIP	0.70 ± 0.24	1.10 ± 0.17	1.20 ± 0.10	1.90 ± 0.69	0.30 ± 0.03	1.4 ± 0.16
Norharman	10.41 ± 0.26	10.51 ± 1.03	11.87 ± 0.93	9.94 ± 0.02	4.06 ± 0.01	11.00 ± 0.01
Harman	4.3 ± 0.12	4.8 ± 0.56	5.7 ± 0.36	4.4 ± 0.0	2.0 ± 0.00	4.6 ± 0.00

Means with different letters are significantly different.

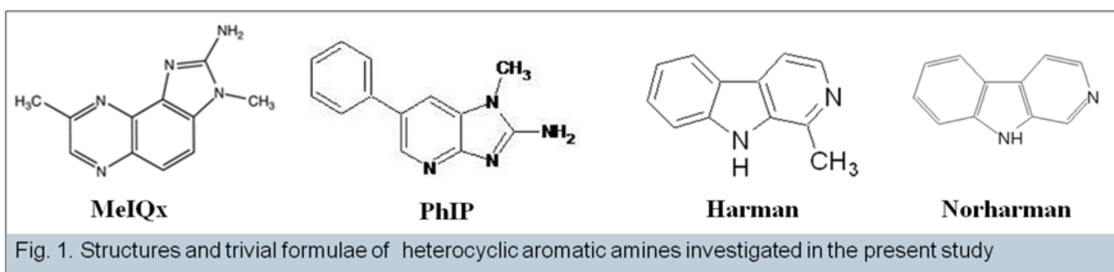
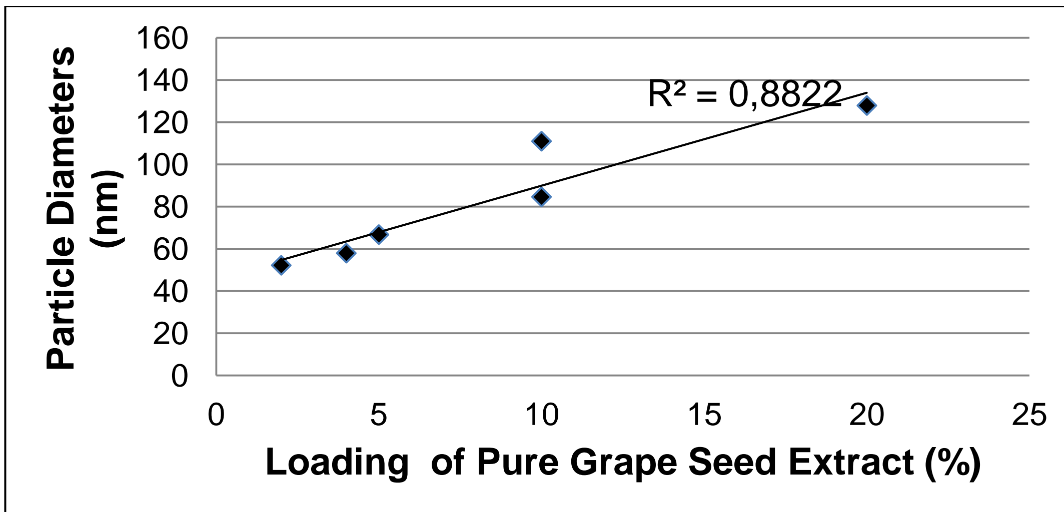
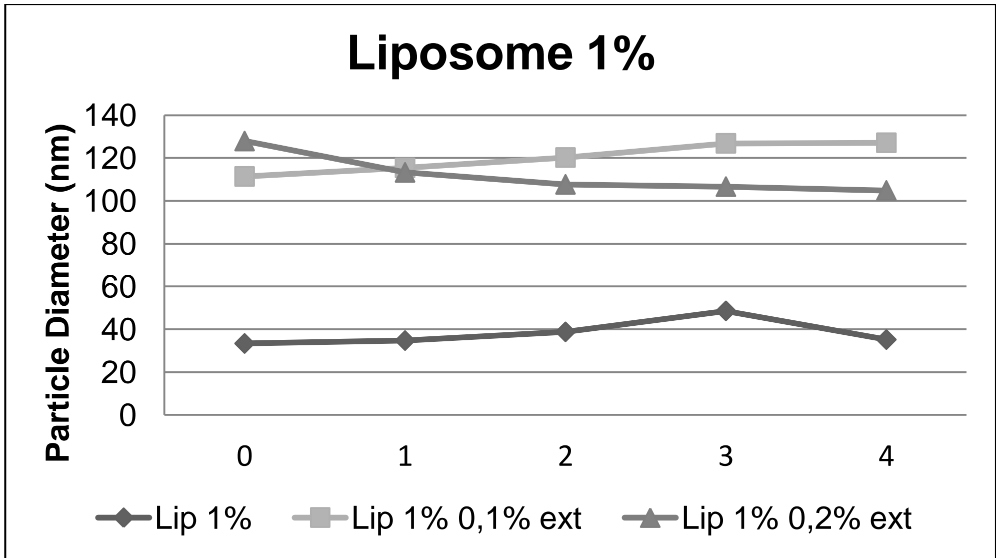


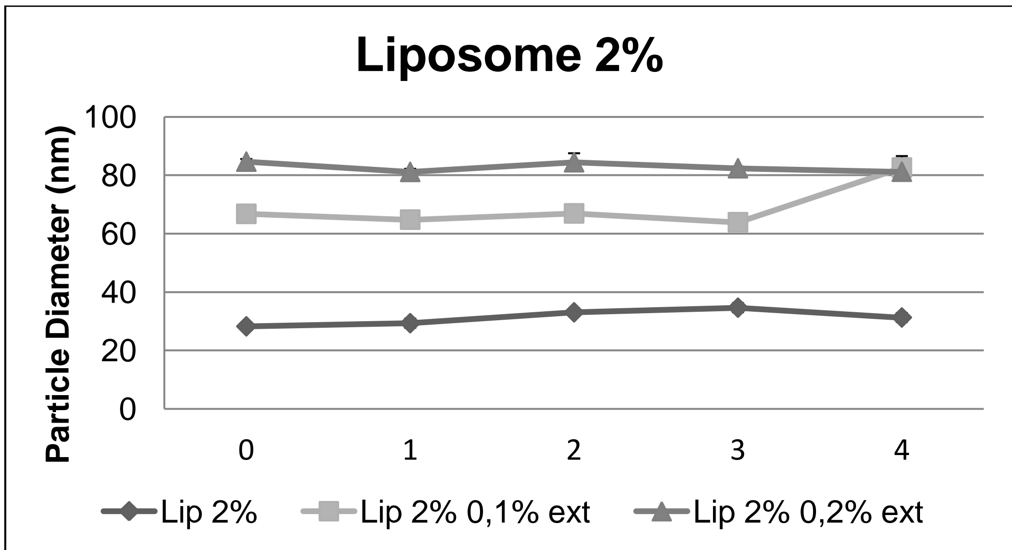
Fig. 1. Structures and trivial formulae of heterocyclic aromatic amines investigated in the present study



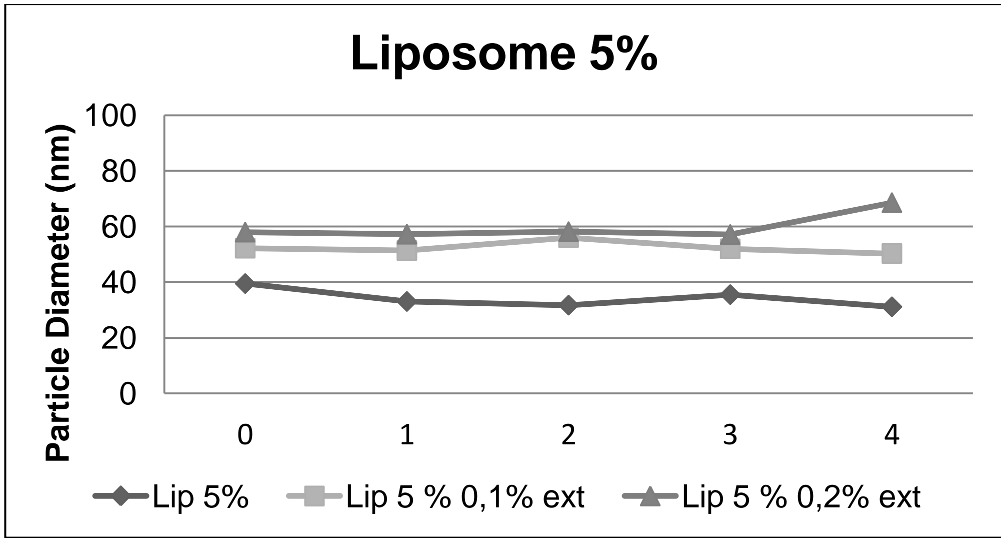
Graph 1. Correlation between particle size and pure grape seed extract (loading)



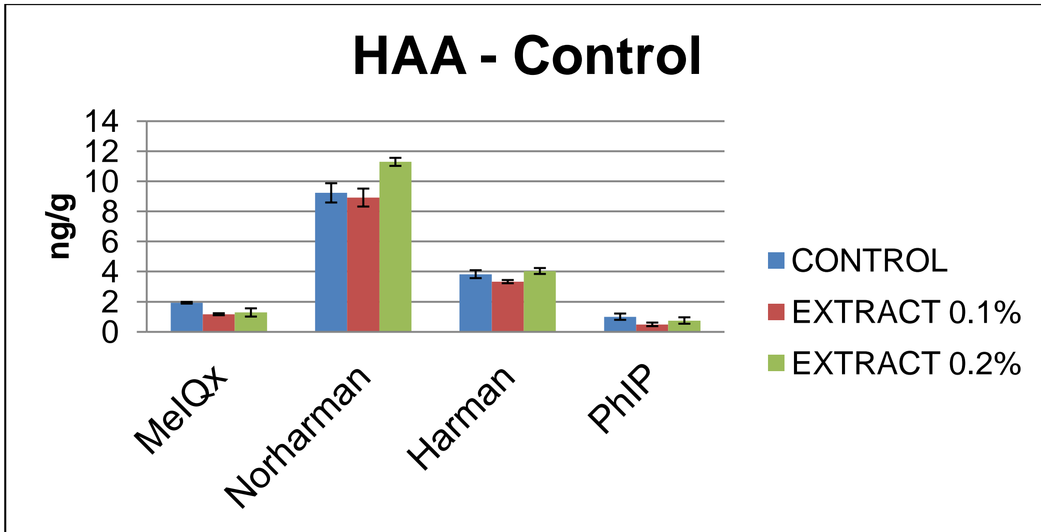
Graph 2: Physical stability of liposomes (1%) during the storage (week)



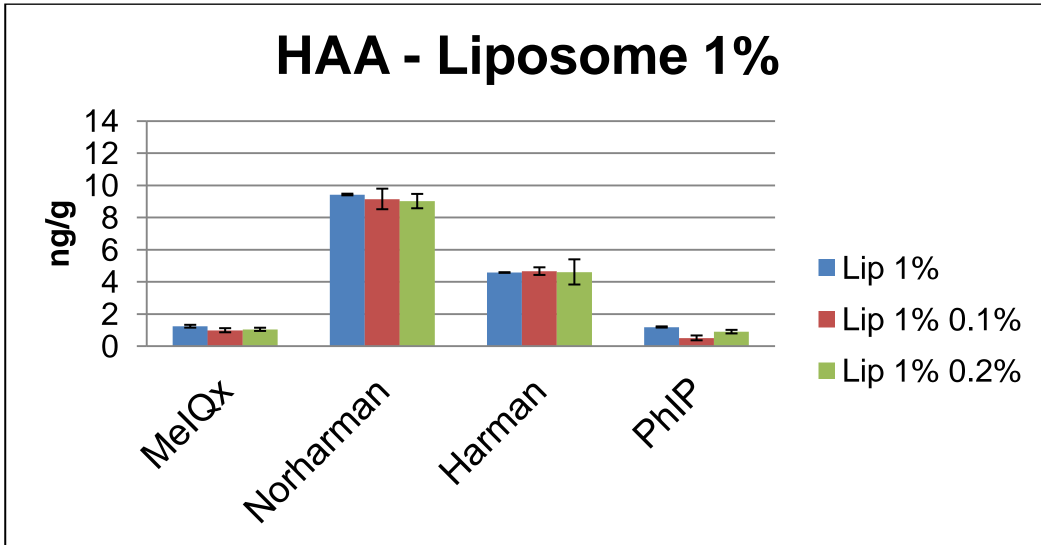
Graph 3: Physical stability of liposomes (1%) during the storage (week)



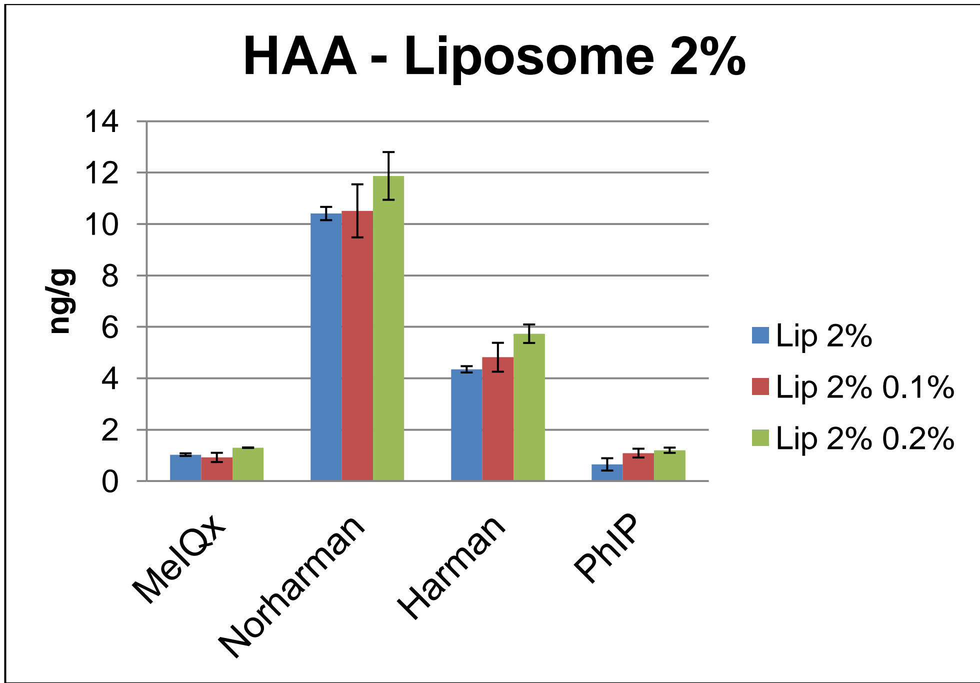
Graph 4: Physical stability of liposome (5%) during the storage (week)



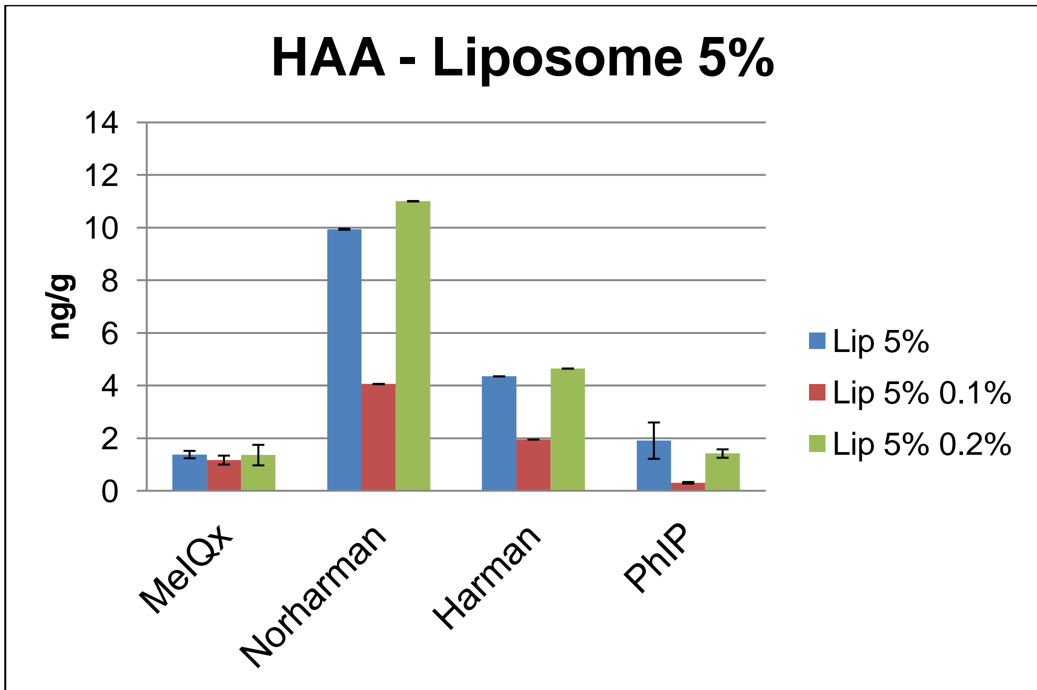
Graph 5: Heterocyclic Amine content (ng/g) detected in the Control trial



Graph 6: Heterocyclic Amine content (ng/g) detected in the Liposome 1% trial



Graph 7: Heterocyclic Amine content (ng/g) detected in the Liposome 2% trial



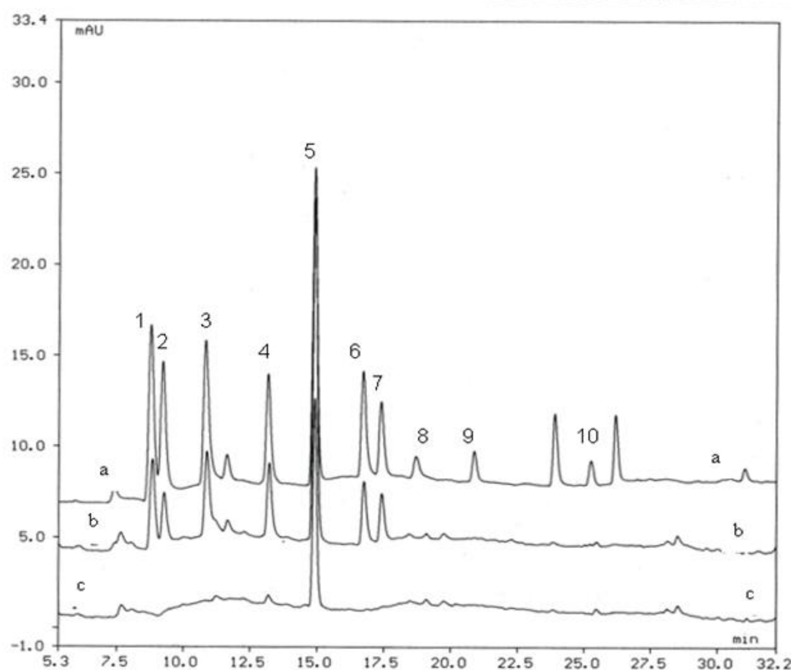
Graph 8: Heterocyclic Amine content (ng/g) detected in the Liposome 5% trial

Figure 2. Fig. 7: HPLC-UV Chromatogram (250 nm) of the polar fraction.

a. Standard Mixture

b. Spiked sample

c. Unspiked sample



1. IQ, 2-amino-3-methylimidazo[4,5-*f*]-quinoline
2. IQx, 2-amino-3-methylimidazo[4,5-*f*]-quinoxaline
3. MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]-quinoline
4. MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline
5. Caffeine
6. 7,8-DiMeIQx, 2-amino-3,7,8-trimethyl-imidazo[4,5-*f*]-quinoxaline
7. 4,8-DiMeIQx, 2-amino-3,4,8-trimethyl-imidazo[4,5-*f*]-quinoxaline
8. Norharman, 9H-pyrido[3,4-*b*]-indole
9. Harman, 1-methyl-9H-pyrido[3,4-*b*]-indole

PhiP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine

Conclusions

The study conducted during the PhD research was focused on the assessment of various thermoxidative degradations in frying oils. To achieve this objective, it was considered appropriate to first proceed with a screening of the major frying oils present in the Italian market, followed by the development of two frying blends (made of vegetable oils), which were subjected to two different experimental frying plans at lab-scale, under controlled and standardized conditions. The same frying blends were also utilized under two different catering frying situations (cafeteria and restaurant). Each frying blend was compared with two reference frying oils.

To evaluate the frying performance of each frying oil and blend, different analytical determinations were carried out (fatty acid composition, total tocopherol content and composition, parameters of hydrolytic and oxidative stability, smoke point, polar compounds, composition of volatile compounds); such analysis were performed before, during and at the end of the frying process.

The first part of project focused on the screening of the major frying oils present in the Italian market evinced the good hydrolytic and oxidative characteristics of all frying blends present on the Italian market. In particular, two oils blend with an elevated percentage of MUFA class in the fatty acid composition exhibited the highest OSI value. The highest percentage of diacylglycerols was displayed by the commercial blends that contained palm oil, which could be mainly correlated to the extraction technology of the latter. The deermination of diacylglycerols level is of outmost importance, as they can affect the oxidative stability, foam production and polar compounds content of the frying oils.

In the second part of this study, the aim of work was to compare the oxidative and hydrolytic stability of a vegetable oil blend (high-oleic sunflower oil:palm olein, 60:40, v/v) with that of palm

olein, during frying under standardized lab-scale and real catering conditions. Firstly, both vegetable oils were utilized for French fries frying under standardized lab-scale conditions. In a second phase, the performance of both vegetable oils was tested in two catering situations (restaurant and cafeteria), where different food products (potatoes, battered vegetables, chicken nuggets and floured fish) and quantities were fried depending on the customer's request. Under standardized conditions, both vegetable oils showed a good oxidative and hydrolytical stability; the differences observed on the free acidity value and diglyceride content were mainly related to the oils' extraction and refining technology. In the restaurant and cafeteria trials, different oil trends were observed during frying, which could be ascribed to the different quantity and/or type of fried food. In these catering conditions, the palm olein displayed a greater oxidative stability than the frying oil blend, which is probably related to the higher content of saturated fatty acids (41%) present in the palm olein as compared to the blend (21%). From the hydrolytic standpoint, the palm olein displayed a diacylglycerol content (11%) that was almost trice as much that of the frying oil blend (4%). However, such diacylglycerol levels remained fairly constant regardless of the frying conditions (time, temperature, amount/type of fried food); this parameter trend is of outmost importance, since diacylglycerols can have a noticeable effect on foam formation and, thus, affect frying oil performance. Considering the overall performance of both oils under standardized lab-scale and real catering conditions, the frying oil blend therefore represents a good alternative to the utilization of palm olein.

The frying performance of a high-oleic sunflower palm olein-blend (high-oleic sunflower:palm olein (55:45, v/v) and palm olein was evaluated under standarized lab-conditions. Several parameters that assess the thermo-oxidative and hydrolytical alterations in frying oil, were determined and monitored for 5 days of intermittent frying conditions; in addition, the tocoferol and tocotrienol content was also evaluated. The results obtained from the analytical evaluation of the two frying oils led to conclude that the selected high-oleic sunflower-palm oil blend (55:45, v/v)

may represent a valid alternative to pure palm olein as frying medium, even though this blend showed a faster increase in some oxidation indices (OSI), mainly due to its higher unsaturation degree. However, the blend displayed a lower hydrolytic degradation, as confirmed by the free acidity and diacylglycerol levels. Moreover, the lower diacylglycerol content may represent a further advantage from the technological standpoint, as it results in less foaming which allows a better handling.

The chemical performance of extravirgin olive oil was compared with those of other vegetable oils, during repeated deep-frying conditions. For this purpose, an extravirgin olive oil (cv. Coratina) and a frying oil blend (high oleic sunflower oil:palm oil, 60:40, v/v) were used, both characterized by a high MUFA level. Standardized frying lab-scale conditions were employed and both hydrolytic and oxidative parameters were monitored for 5 days of intermitting frying; the alteration picture was completed with the determination of the total tocoferols and tocotrienol content.

The stability and usage of a special marinade, made of grape seed phenolic extracts encapsulated in liposomal systems, was tested on the formulation of beef patties. The ultimate objective was to evaluate the ability of phenol-enriched liposomes to inhibit the formation of heterocyclic aromatic compounds generated by pan-frying. Liposomes were prepared from soy lecithin (1%, 2% and 5%). Two extract solutions (0.1% and 0.2%) were assayed. The liposomes at different concentration of grape seed extract were used as marinades the beef patties. Polar and non polar heterocyclic aromatic amines were analyzed. For each liposomal preparation with and without extract, the diameter was measured and monitored for five consecutive weeks. The differences in particle diameter between the control (without extract) and liposomes with different extract concentrations already suggest that the polyphenolic compounds had been incorporated in the liposomal bilayer, since liposomes with extract were larger than liposomes without it (40 nm and 80 nm, for control and 0.1% extract in liposomes, respectively). During the five weeks of storage, particle diameters remained virtually unchanged, a sign of the high stability of the liposomal

preparation. Analysis of the polyphenolic content showed that most of the polyphenolic compounds had been incorporated in the bilayer membrane. Analysis of heterocyclic aromatic amines formed in beef patties showed the positive effect of the presence of encapsulated polyphenolic compounds, i.e. formation of heterocyclic aromatic compounds amines in patties after frying was significantly reduced. MeIQx and PhIP were the most abundant compounds in the polar fraction. The co-mutagenic Norharman and Harman were also found in the apolar fraction. In general, pretreatment of patties with grape seed extract containing liposomes reduced formation of heterocyclic aromatic amines during pan-frying. Results showed that liposomes can entrap phenolic compounds without loss of inhibitory effects on the formation of heterocyclic aromatic compounds amines

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