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# Improving the oxidative stability of edible oils: current trends, challenges and solutions

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***Abstract***

This work represents an overview on literature data to reveal the issues generated by lipid oxidation, the associated causes as well as the current trends, challenges and solutions to enhance the oxidative stability of edible oils. The lipid oxidation represents one of the main reasons for deterioration of vegetable oils and lipid-containing foods. In results, a wide spectrum of volatile or non-volatile components are generated, including free fatty acids, aldehydes and ketones, alcohols, hydrocarbons, trans isomers, cyclic and epoxy compounds. The use of natural extracts as antioxidant agents instead of the synthetic ones has been very popular because of their antioxidant activity. Recent researches has highlighted the need to identify new natural sources of antioxidants to be uses as food additives and also to assess their potential to inhibit the lipid oxidation. This review indicates that there are many possibilities to use natural antioxidants to improve the oxidative stability of freshly consumed vegetable oils as well as those used as a heat transfer medium in various food applications. The summarized information reveals the effectiveness of antioxidant plant materials in the form of natural extracts or powders from medicinal plants, culinary herbs, spices and food processing by-products to inhibit the lipid oxidation, which recommends them as natural antioxidant agents for the vegetable oils industry. This study may be useful for the oils industry for replacing the synthetic antioxidants with natural formulas, able to inhibit the oxidative lipid degradation.

***Keywords***: oxidative stability, lipid oxidation, edible oils, antioxidant plant materials, natural extracts or powders

## 1. Introduction

It is unanimously acknowledged that, in adition to the genetic factor, a series of diseases specific to contemporary civilization are closely related to the quantity, and especially to the quality of consumed food products. The nutrition policy adopted in various countries is based on the promotion of new foods with very high biological potential, obtained either from raw materials with a valuable content in biologically active compounds, or through a processing that increases their nutritional potential.

The designing and development of products that provide health benefits is relatively recent and comes as a result of accepting the idea that food has a key role in the prevention and treatment of mmany diseases. The production of foods with superior physico-chemical, microbiological and nutritional characteristics is currently a major goal of the local and world food industry. The food producers face situations in which, even if they start from safe raw materials, the stages they go through in the flow of the food chain, make the finished products have inferior physico-chemical and nutritional characteristics, even potentially toxic.

In this spirit, the addition of natural biactive ingredients is more than a welcome choice.

Lipid oxidation represents a serious problem facing some sectors of the food industry due to the growing trends of enriching foods in

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polyunsaturated fatty acids, but also the widespread use of vegetable oils in various food recipes. In response, there are recorded a large number of unwanted changes in flavor, texture and nutritional value of lipid-containing foods [1, 2].

Also, in the food industry there are many products obtained by frying in vegetable oil. The edible oils undergo a series of chain degradation reactions during high-temperature processing, generating potentially toxic compounds that reduce the organoleptic attributes and nutritional value. The same reactions may occur during the long time storage, being closely related to the storing temperature, the presence of oxigen and the exposure to radiation [3, 4].

The primary products of lipid oxidation, hydroperoxides, have no odor, while the secondary products of oxidative degradation are often volatile, with a strong odor and an unpleasant taste associated with rancidity. The resulting peroxides may react with other constituents of oils or decompose into secondary products (aldehydes, ketones, hydroxy acids). Peroxides, free radicals as well as the generated secondary products can react with proteins and vitamins causing a loss of nutritional value. But the most important risk is the ingestion of lipid oxidation products, because they present enormous toxicological risks, and in the case of long-term use they can cause degenerative pathologies such as arteriosclerosis, cancer, etc. [5].

Due to the toxicological risks and nutritional losses associated with the process of oxidative degradation of fatty acids in vegetable oils, as well as the need to ensure optimal sensory quality of lipid-containing food products, it is absolutely necessary to study the ways to slow down the lipid oxidation in edible oils.

There are numerous reasons that led to this study, but the most important is that related to the consumer safety. Polyunsaturated fatty acids (PUFAs) from the composition of vegetable oils and lipid-containing foods are susceptible to oxidation generating a series of unstable reaction products such as peroxides, peracids, malondialdehyde and 4-hydroxynonenal, that can form adducts with some biomolecules such as proteins, deoxyribonucleic acid and phospholipids with short catenary chain. The rate of lipid oxidation can be effectively reduced by addition of antioxidant agents.

Synthetic antioxidants are widely used in the vegetable oils industry, but consumers' reluctance and concerns about their toxicity have led to a continuing search for natural alternatives [1].

As a result, some plant materials such as medicinal plants, culinary herbs and spices, various by-products resulting from food processing have been investigated as potential natural antioxidants due to their high contents of bioactive compounds with antioxidant and antimicrobial properties [6-9].

Research performed so far has shown that natural antioxidants obtained from various plant materials can be used as alternatives to synthetic antioxidants due to their equivalent or even greater effect on inhibiting lipid oxidation [10-12].

The natural extracts obtained from these plant materials are rich in natural antioxidants belonging to polyphenolic compounds class, such as flavonoids, tannins and phenolic acids. These compounds have antioxidant, anti-inflammatory and anti-cancer activity. The addition of these extracts to food systems can improve the flavor of products, delay the damage caused by lipid oxidation, inhibit the growth of microorganisms and also play an important role in reducing the risk of disease [13-15].

The above mentioned considerations highlight the opportunity and importance of studies related to the enrichment of oils and fats, but also of lipid-containing foods, with bioactive compounds by incorporating antioxidant plant materials in the form of extracts or even powders obtained from the plant materials concerned. In this purpose, we started from the hypothesis that the natural formulas from various plant materials with a high content of specific phytochemicals, will have synergistic antioxidant and antimicrobial effects, will inhibit the development of pathogenic and altering bacteria and also, their addition will inhibit the evolution of oxidative processes.

As a result, the studies developed in recent years have been focused on the improvement of the oxidative stability of oils and fats by exploiting the antioxidant potential of some valuable plant origin sources. In this line, our work will address the current trends, challenges and solutions to improve the oxidative stability of freshly consumed vegetable oils as well as those used as a heat transfer medium in various food applications.

## 2. Issues generated by lipid oxidation

Lipid oxidation is a general term used to describe a complex sequence of chemical changes, which results from the interactions of various lipids with oxygen and/or active oxygenated species (prooxidants). As a consequence of this phenomenon, there may be a rancid odor, a change in color, which negatively affects the sensory qualities of these products. The nutritional value of oils subject to oxidative degradation of the lipid fraction can also be significantly affected [16, 17].

The lipid oxidation is associated almost exclusively with the oxidation of unsaturated fatty acids by a self-oxidation mechanism. The reaction proceeds through the radical chain mechanism and has three distinct steps: initiation (radical formation process), propagation (transfer reaction of free radicals, thus, the number of reactive compounds will be multiplied) and termination, when the reactive compounds previously generated will degrade or will react with each other in order to give non-reactive compounds (for example, two free radical species react with each other leading to a stable, non-radical adduct). A particular example of a termination reaction is the reaction that takes place between a peroxy radical and an antioxidant compound. Lipid oxidation occurs especially in the case of vegetable oils and food products rich in polyunsaturated fatty acids, as well as in the food lipids used in various food thermal applications or with a considerable surface in contact with air. During this process, a series of more or less stable compounds are generated, which cause the appearance of an unpleasant odor, the decrease of the nutritional value of the product, the appearance of the rancid phenomenon, that negatively impact on the consumers' acceptance of food products [18].

The primary products of lipid oxidation, hydroperoxides, have no odor, while the secondary products of oxidative degradation are often volatile, with a strong odor and an unpleasant taste associated with rancidity [19].

The limit of perceived rancid odor can vary considerably, but even in the case of minor development of the oxidation process, it can induce unacceptable changes in food products flavor. This has been particularly noticeable in the case of commercial deodorized oils and fats containing polyunsaturated fatty acids of the n-3 series, the products of their oxidation having a very low limit of perception. The resulting peroxides may react with other constituents of oils or decompose into secondary reaction products such as aldehydes, ketones and hydroxy acids. The generated peroxides, free radicals as well as the secondary products can react with proteins and vitamins causing important losses of nutritional value. But the most important risk in the case of lipid oxidation is represented by the ingestion of lipid oxidation products, because they present enormous toxicological risks, and in the case of longer-term use they can cause degenerative pathologies such as arteriosclerosis, cancer, etc. [20].

The resulted peroxides and carbonyl products have a toxic potential due to their electrophilic character as well as the fact that the lipid oxyl and peroxyl radicals that occurr in the different reaction stages are highly oxidizing species that can damage proteins and deoxyribonucleic acid, ultimately leading to a number of cardiovascular diseases and cancer [21].

The nutritional consequences of lipid oxidation consist of a decrease in the content of n-3 and n-6 PUFAs, oxidation of vitamin A, carotenoids, vitamin D and folic acid. The losses of linoleic and linolenic acids are in most cases insignificant, because they are present in significant quantities in lipid-containing foods. The losses of acids with a higher degree of unsaturation, such as arachidonic and docosahexaenoic fatty acids involved in the protection against various pathologies, can be considerable as a result of lipid oxidation. Also, the ingestion of lipid oxidation products results in significant associated toxicological risks [22].

Taking into account these nutritional consequences, the *in vivo* lipid peroxidation is considered as the main cause of the development of severe pathologies such as cancer, diabetes, cardiovascular diseases, rheumatoid arthritis, etc. Peroxyl radicals can interact with deoxyribonucleic acid causing important changes in the structure of nitrogenous bases by reactions of deamination, oxidation and hydroxylation. These transformations appear to have an important contribution to the development of carcinogenesis [23-25].

The secondary products of lipid oxidation can cause liver pathologies. Aldehydes, due to their high reactivity and toxicity, also reduce the activity of various enzymes, thus blocking the protein synthesis. It has been proven that the generated aldehydes block the action of macrophages and stimulate the *in vivo* production of thrombin. Also, malonaldehyde shows a significant carcinogenic and mutagenic activity. *In vivo* research conducted on animals has shown that the administration of vegetable oils with a high degree of lipid degradation by oxidative processes caused various pathological responses, the most important being the increase of kidney and liver mass, changes in the composition of fatty acids in the adipose tissue, changes in the level of prostoglandins, severe intestinal tract irritations, developmental delays, etc. [23-25].

The oxidation of the lipid fraction has been shown to induce some depreciation of the quality of vegetable oils and lipid-cointaining foods. It has been proven that the oxidative by-products can react with the proteins that accompany lipid fraction in food, reactions that also lead to structural and compositional changes that affect the functional properties of these foods [26].

Lipid oxidation is responsible for changes in the aroma and taste in vegetable oils due to the appearance of secondary reaction products. Currently, there is a great interest in obtaining vegetable oils with a high content of essential fatty acids (linolenic acid, linoleic acid and arachidonic acid). A vegetable oil is considered more valuable from a nutritional point of view, as its content of polyunsaturated fatty acids is higher, but in the same time, it is more easily affected by oxidative processes [27].

***Factors influencing the oxidative stability***

Lipid containing foods have in their composition a wide variety of fatty acids that differ in physical and chemical properties and susceptibility to oxidation. There are many factors that influence the oxidative stability of food such as temperature, surface area, oxygen concentration, transition metals and degree of unsaturation.

***Fatty acid composition.*** The rate of lipid oxidation is affected by the number, position and geometry of the double bonds. Hydrogen atoms adjacent to the double bonds are most susceptible to abstraction during the propagation phase of lipid oxidation. The more fatty acids a double bond contains, the faster the oxidation will occur. In addition, *cis* fatty acids are oxidized more easily than *trans* isomers [28].

***Oxygen concentration.*** When oxygen is present in low concentrations, the oxidation rate is approximately proportional to the oxygen concentration. If the oxygen level is high, the oxidation rate is independent of the oxygen concentration. Since the addition of oxygen to the alkyl radical is a limited diffuse reaction, most of the oxygen must be removed from the system to inhibit the lipid oxidation. Vacuum conditions are often required to reduce the oxidation because the oxygen removal can be difficult due to its higher solubility in oil than in water [28].

***Temperature.*** As the temperature rises, the oxidation rate will increase. At room temperature, the self-oxidation of saturated fatty acids is slow. At high temperatures, saturated fatty acids can suffer considerable oxidation. However, with most vegetable oils, rising temperatures can reduce the solubility of oxygen, which in turn has the ability to slow down the oxidation rate [28].

***Surface area.*** The rate of lipid oxidation will increase closely related to the surface of lipids that is exposed to oxygen and prooxidants, as can be seen in most vegetable oils and muscle tissue. In oil-in-water emulsions, the surface does not appear to influence the rate of lipid oxidation because the surface is already large [29].

***Transition metals.*** Traces of heavy metals such as iron and copper are usually present in edible vegetable oils. These metals come from animals, plants, soil, dust, metal equipment used during processing and storage. The concentration at which these traces of metals occur in natural lipids is a major factor that causes oxidative damage to lipids. Even at concentrations below 0.1 ppm, the presence of transition metals can decrease the induction period and increase the oxidation rate. Transition metals support the formation of free radicals through the abstraction of hydrogen and the decomposition of hydroperoxides, which leads to an acceleration of the lipid oxidation reaction. The abstraction of hydrogen from an unsaturated fatty acid leads to the formation of a single alkyl radical [30].

***Enzymes and singlet oxygen.*** Lipoxygenases are found in plants and animal tissues and they support the lipid oxidation process by catalyzing the formation of lipid hydroperoxides. Singlet oxygen is formed in the presence of light and it directly reacts with unsaturated fatty acids to form the lipid hydroperoxides [31].

## 3. Current trends, challenges and solutions

Given the toxicological risks and associated nutritional losses, as well as the need to ensure optimal sensory quality of food, it is absolutely necessary to identify some ways to inhibit the lipid oxidation in vegetable oils. Because the oxidative processes in vegetable oils are initiated by free radicals, the simplest and the most convenient solution to inhibit or delay these processes is represented by the addition to the oil of substances with antioxidant capacity. In general, an antioxidant is defined as any substance that, when is present in a low concentration compared to an oxidizable substrate, can significantly delay or inhibit the oxidation of that substrate. In addition, to inhibiting or delaying the lipid oxidation, the antioxidants are substances that extent the shelf life of oils by protecting them from oxidative damage [32].

The ability of phenolic compounds to inhibit lipid peroxidation is well known, which has led to the introduction of synthetic antioxidants such as butylate hydroxyanisole (BHA), butylate hydroxytoluene (BHT), propyl-gallate (PG) and tertbutylhydroquinone (TBHQ) in lipid-containing foods [33].

Although they are still widely used today, some physical properties of synthetic antioxidants, such as high volatility, thermal instability at high temperatures as well as very strict legislation on food additives and the consumer preference for natural products, have led to a obvious search for natural compounds with antioxidant properties, as evidenced by the huge variety of plant species (fruits, vegetables, seeds, herbs) investigated by scientists [34, 35].

That is why today we are witnessing major changes in the food industry: the replacement of synthetic antioxidants with natural antioxidants and the use of antioxidant-rich extracts instead of a monoantioxidant, due to their multitarget effect [36-38].

The studies presented in the literature give a great importance to natural substances with antioxidant activity, derived from spontaneous flora, which are used as food additive in the form of standardized extracts, to express their protective function against ROS [39].

Although at this stage of technology development, the natural substances with antioxidant properties are not yet competitive with the synthetic ones, they could be a promising alternative to them, because they are obtained from cheap and renewable raw materials. Also, when are added to a product, they are able to express their multifunctional properties, to maintain a balance between the pro and antioxidant capacity of each food but also to provide protection at the cellular or tissue level against some microorganisms.

At global level, there is a great interest in wine industry by-products capitalizatio, because a considerable amount of waste and by-products annually result from this sector [40, 41].

The by-products resulting from the grapes vinification as well as from the wine conditioning represent approximately 25% of the quantity of grapes subjected to processing.

Due to its high content of polyphenolic compounds, in particular proanthocyanidins, the grape pomace represents a valuable and underused source of biologically active compounds with potential applications in the food, cosmetics and pharmaceutical industries. The antioxidant activity of polyphenolic compounds in natural extracts depends on that part of the polyphenolic molecule with important electron donation properties. The antioxidant effect results both from the activity of scavenging free radicals and from the property of chelating with metals, the first being the predominant one. Besides to the antioxidant effect, these compounds also have other biological properties such as anti-inflammatory, anticarcinogenic and antimicrobial [42, 43].

For these reasons, the addition of natural active ingredients obtained in the form of natural extracts from pomace or its main fractions, grape seeds and skins, is an appropriate choice. Unlike other natural antioxidants in the category of tocopherols, the phenolic compounds present in the skin and grape seeds, as the main constituents of pomace, are stable at high temperatures, do not give a bitter taste to food and also they are odorless. By assessing their antioxidant activity using the soybean oil as a substrate has shown that they are more effective in inhibiting fatty acid oxidation than BHA and BHT. The antioxidant activity of these extracts remained unchanged, even after 6 months of storage [44, 45].

In addition, microbiological studies performed on these extracts revealed that they have antimicrobial and antifungal activity [44, 46].

The antioxidant activity of grape seeds and skins extracts is influenced by the polarity of the solvent used as the extraction medium. It was proven that the ethanol extracts have been shown to be the most effective to delay the oxidative degradation of sunflower oil, comparatively with extracts obtained by using hexane and ethyl acetate as extraction medium for polyphenolic compounds. These results are strong arguments for obtaining and using natural extracts from grape pomace to inhibit/delay the oxidative degradation of some lipid substrates.

The studies performed in this field revealed that the ultrasound increases the extraction efficiency of antioxidants from medicinal plants and reduces the extraction time to the same extraction temperature even in the case of using weak solvents under normal extraction conditions. Ethanol, under extraction conditions combined with ultrasonication, has been shown to be as effective as ethyl acetate and butanone. Also, by using dried plant materials, the yield in active principles was higher than in the case when the medicinal plants were used fresh [16, 47]. These findings open new perspectives on the use of herbal extracts in the food and pharmaceutical industry.

Recent studies have shown that a wide range of plants such as sage, rosemary, basil, oregano and marjoram contain polyphenolic compounds that give antioxidant potential to the extracts. These studies revealed that the natural extracts from these plant materials are important sources of natural antioxidants belonging to the polyphenols class. Polyphenolic compounds with antioxidant effect from the herbs and spices are flavonoids, such as phenolic acids and 3-ol flavan monomers (catechin, epicatechin, galocatechin, epicatechin 3-O gallate, dimers, trimers and polymers of procyanidins) [10, 48].

The kinetic of the peroxides accumulation in sunflower oils was monitored in the presence of different concentrations of natural extracts obtained from lemon balm (*Melissa officinalis* L.), mint (*Mentha piperita* L.), *Mentha spicata* L., basil (*Ocimum basilicum* L.), oregano (*Origanum vulgare* L.) and garden thyme (*Saturejae hortensis* L.) using hexane, ethyl acetate and ethanol for extraction. It was found that the ethanolic extracts were the most effective in delaying the autooxidation process. The most active was the garden thyme extract followed by the mint and rhubarb extracts.

These studies show that some culinary spice plants are recommended as a source of natural antioxidants to stabilize the polyunsaturated lipid systems [49, 50].

The chemical and sensory characteristics of extra virgin olive oil flavored with pepper, garlic, oregano and rosemary were determined during 7 months of storage. The results showed that the both spices and culinary spice plants improve the stability of extra virgin olive oil. These flavoring methods can be applied immediately in practice, the process being adapted according to consumer preference. In this way, new products are obtained with high added value due to the antioxidant and nutritional power of spices [51].

The ability of extracts from culinary herbs and spices to protect alpha-tocopherols in sunflower oil againt degradation has been tested. The extracts of rosemary, thyme, curcumin, sage, oregano and cumin, applied to a level of 2 g/kg oil were found to delay the rancidity and protect the alpha-tocopherols. To a limited extent, the garlic clove extract also provides protection against oxidation, while the corinadru and cardamom extracts were acted as pro-oxidants. In the case of using thyme extract, the log period of the induction process, as an indicator of the rancidity delay, was directly proportional to the temperature (85-100°C). In the case of the use of thyme, rosemary and sage extracts, the increase in the conservation of alpha-tocopherols was directly proportional to the concentration of plant extracts and was effective even at 0.1 g/kg oil. As the period of rancidity increased, the stability of tocopherols has improved. Other experiments in which turmeric extracts were used showed that, the conservation effect of turmeric is not due to the antioxidant compounds present in the extract, even if the rancidity of the respective oil has been delayed. When the extracts of herbs or spices were applied in the mixture, the phenomenon of synergism was observed, while the single use of some extracts had a slight inhibitory effect. This mode of action can most likely be explained by antiradical activity rather than by the generation of a singlet oxygen [52].

The analysis of rosemary (*Rosmarinus officinalis* L.) extracts revealed the existence of four diterpenoid components such as carnosol, rosmanol, isorosmanol, and rosemaridiphenol. Unlike other natural antioxidants in the category of tocopherols, catechins or gallates, these compounds are stable at high temperatures, do not impart a bitter taste to food and are odorless. The investigation of their antioxidant activity using soybean oil as a substrate has shown that rosemaridiphenol and rosmanol are more effective in inhibiting the glyceride oxidation than BHA and BHT. In addition, microbiological studies performed on these compounds revealed that they all show antimicrobial and antifungal activity [44].

The culinary herbs as well as the medicinal plants could be used as natural food ingredients to replace the synthetic antioxidants. By extraction of a mixture from some medicinal plants belonging to the *Lamiaceae* family, such as rosemary, lemon balm, self-heal and hyssop were obtained two natural antioxidant preparations, in liquid and powder form. The total polyphenol content of the preparations was very high. The main active compound identified in these preparations extracts is supposed to be the caffeic acid derivative. The obtained formulas acted as primary and secondary antioxidants, by chelating the transitional metal ions and inhibiting the linoleic acid autoxidation. The two preparations exhibited strong antioxidant property that was unchanged after six months of storage. Based on the in vitro studies, these products are suitable to be used as antioxidant ingredients instead of synthetic antioxidant agents in lipid-containing food products [46].

In the last decades, a new approach has been observed, in order to obtain extracts rich in antioxidant compounds, used the edible vegetable oils as the extraction medium. Results reported in studies that deal with assessing the ability of oily extracts to inhibit the oxidative degradation processes in model systems have shown that although the efficacy of ethanolic and methanolic extracts from medicinal plants is slightly higher than that of oily extracts, this new extraction technique is safer, in terms of consumer health. The antioxidant activity of these oily extracts was comparable to that of the n-hexane extracts. In addition, the inhibition of lipid oxidation by the oil extracts was superior to tocopherol [53].

Because many oil seeds compounds already have demonstrated nutritional benefits, there are great opportunities for using them to develop new formulas of functional vegetable oils. Edible oils containing high levels of bioactive compounds could have a substantial impact on human health, considering the high amount of oils, freshly used, or as a heat transfer medium for cooking. In the vegetable oils industry it was also investigate the possibility to develop functional oils by fortification of ordinary edible oils with additional quantities of some specific functional ingredients. This method allows the addition in edible oils of precise amounts of components with beneficial properties, while at the same time, the original sensory attributes of the products, that consumers already know, are maintained [54].

Compared to conventional vegetable oils, the technology of newly designed products, enriched in antioxidants added in the form of standardized natural extracts obtained by non-aggressive techniques, aims at minimal intervention of synthetic chemical food additives, optimizing nutritional and sensory characteristics, preventing nonspecific odors during the processing and storage, the prevention of the oxidation of tocopherols and other compounds susceptible to ozidation, etc.

Another way to increase the amount of beneficial compounds in edible oils would be represented by developing a gentler production technology in order to retain a more level of functional ingredients that naturally exist in the oil seeds. The ois obtained in this way are cloudier, have an unusual colour or a stronger and more specific taste, but in time, the consumere will be accomodates with the new features of these products with more health benefits compared with the conventional vegetable oils.

This study may provide additional information on how extracts rich in natural antioxidants added to vegetable oils may inhibit or delay the oxidation of lipids, which may be a challenge for both food industry researchers and technology engineers.

## 4. Conclusions

Lipid oxidation represents a serious problem facing some sectors of the food industry due to the trend of enriching foods in polyunsaturated fatty acids, but also the widespread use of vegetable oils in various food recipes. Synthetic antioxidants are widely used in the oils industry, but consumers' reluctance and concerns about their toxicity have led to a continuing search for natural alternatives. The antioxidant plant materials in the form of natural extracts or powders obtained from medicinal plants, culinary herbs, spices and food processing by-products can be incorporated into oils or lipid-containing foods to inhibit or delay the oxidative degradation processes. These materials can be added singly or in a mixture in order to enrich the vegetable oils in natural bioactive compounds with antioxidant properties, in this way decreasing or avoiding the addition of synthetic antioxidants. Vegetable oils containing high levels of bioactive compounds could have a substantial impact on human health, considering the high amount of oils, freshly used, or as a heat transfer medium for cooking.

**Compliance with Ethics Requirements.** Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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