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OXIDATION OF LIPIDS IN FOOD

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An overview of lipids oxidation in foods, its main aspects and implications for the customer has been outlined in a concise form.

Three different mechanisms of fatty acid oxidation yielding different products are described: autoxidation, photo-oxidation and lipooxygenase action. Oxidation of sterols and fat-soluble vitamins (named A, D, E and K), all isoprenoic compounds, as a part of unsaponificable fraction of lipid are included to review. It is indicated that relatively little information exists on oxyphytosterols. The contents of these compounds in food products are presented.

The crucial significance for the sensory aspect of food quality is ascribed to volatiles from lipid oxidation products. Some volatiles with low odour threshold are characterized.

Nutritional problem of lipid oxidation products and products obtained by interactions with other food components is discussed. Use of antioxidants as important factors in reducing the risk of chronic diseases and its economical aspects is discussed. The need for search of natural antioxidant, commonly perceived as safe is stressed.

Last part of review covers the methods for the assessment of lipid oxidation. Classic methods for the determination of lipid stability, for the measurement of lipid oxidation are described. Also chromatographic analyses of volatile compounds generated in the lipid oxidation process included static, dynamic head space and SPME are discussed. The novel approaches to the analysis of these volatiles such as chemometrical methods and electronic noses are also presented.

INTRODUCTION

Oxidation is one of the most important processes occurring in food systems. It affects many interactions among food constituents, leading to both desirable and undesirable products. Food lipids are foods components that are very susceptible to oxidation processes, therefore oxidation reactions are one of the major sources of deterioration that occurs during manufacturing, storage, distribution and final preparation of foods.

Lipid oxidation products are ubiquitous in foods, although much variation exists in their kind and levels present. Although levels of these compounds are generally low, the problem of lipid oxidation severely compromises the quality of some food products and limits the shelf-life of others. All foods that contain lipids, even at a very low level (<1%), are susceptible to oxidation, leading to rancidity. Deleterious changes in foods caused by lipid oxidation include not only loss of flavour or development of off--flavours, but also loss of colour, nutrient value, and the accumulation of compounds, which may be detrimental to the health of consumers.

One of the most effective ways of retarding lipid oxidation in foods is to incorporate antioxidants. They work by a variety of different mechanism, including control of oxidation substrates (lipids and oxygen), control of prooxidants, and inactivation of free radicals. Much interest has developed recently in naturally-occurring antioxidants, which are presumed to be safe since they occur in plants and plant foods. The appeal in application of natural antioxidants as foods additives is raised because of their potential health benefits. This is of special importance when plant extracts rich in phenolic components are applied as foods stabilizer. Endogenous plant antioxidants are capable of inhibiting lipid peroxidation in foods and offer protection against oxidative damage to membrane functions in biological systems. Lipid peroxidation *in vivo* and dietary lipid oxidation products have been proved to be the primary cause of many diseases, *i.a.* atherosclerosis cancer to name the most important, and aging process. Medical sciences' researchers are becoming aware of the value of preventive therapies. Terms like functional foods, designer foods, therapeutic foods, nutraceuticals and the like are widely used in food industry and human nutrition.

Analysis of lipid oxidation products in foods is difficult because of their complex nature, instability, large quantities of interfering substances in foods, and the sophistication and sometimes a lack of specific and adequate analytical

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methods. The presence of natural antioxidants in food systems, being commonly the mixture of compounds of varying mechanism of action, complicates the evaluation of the oxidative status of food lipids. Methods to determine the extent of oxidation can be ranked on the basis of their usefulness in predicting the stability, shelf-life and consumer acceptability. Still sensory analysis is given the closest approximation compared to consumers' approach. Chromatographical methods based on the analysis of specific compounds, including volatiles, chemical indices of oxidation, extent of oxygen absorption are useful in assessing the extent of oxidation, however a search for novel methods based on chemometry and electronical sensing develops rapidly and hopefully will lead to advancement in the knowledge of lipid peroxidation and its influence on food quality.

MECHANISM OF LIPID OXIDATION

It has been recognized as three different mechanisms, yielding different oxidation products: a free radical mechanism, photo-oxidation and process related to lipoxygenase activity.

Autoxidation is a spontaneous reaction of molecular oxygen with lipids, leading to oxidative deterioration. It proceeds by a free radical chain mechanism involving three steps [Frankel, 1985; Hamilton *et al.*, 1997, Gordon, 2001]:

(1) *initiation step* – homolytic hydrogen atom abstraction from a methylene group that leads to alkyl radical ($R^{\bullet}$) formation;

$$RH \xrightarrow{\text{initiator}} R^{\bullet}$$

(2) *propagation step* – formation of peroxy radicals ($ROO^{\bullet}$) able to react with unsaturated fatty acids and form hydroperoxides (ROOH);

$$R^{\bullet} + O_2 \underset{}{\overset{\text{fast}}{\rightleftharpoons}} ROO^{\bullet}$$

$$ROO^{\bullet} + RH \xrightarrow{\text{slow}} ROOH + R^{\bullet}$$

(3) *termination step* – formation of non-radical products by interaction of $R^{\bullet}$ and $ROO^{\bullet}$.

$$\left.\begin{aligned} R^{\bullet} + R^{\bullet} &\longrightarrow RR \\ R^{\bullet} + ROO^{\bullet} &\longrightarrow ROOR \\ ROO^{\bullet} + ROO^{\bullet} &\longrightarrow ROOR + O_2 \end{aligned}\right\} \text{NON-RADICAL PRODUCTS}$$

where: $R^{\bullet}$ – fatty acid radical; ROOH – fatty acid hydroperoxide; $ROO^{\bullet}$ – peroxy radical.

Induction period causes very little changes in lipids, after that lipid deterioration is fast and off-flavours become noticeable. Hydrogen abstraction from unsaturated fatty acids becomes selective for the most weakly bound hydrogen. The ease of hydroperoxidation depends on the number of double bonds present [Frankel, 1985]. In monoeic acids, the most labile are hydrogen atoms on the carbon atoms adjacent to the double bond. In polyunsaturated acids, the most susceptible to abstraction of hydrogen are methylene groups between two double bonds. The radicals formed are not stable and the abstraction is followed by electron rearrangement to form conjugated dienes [Belitz & Grosch, 1999].

According to the classical mechanism of autoxidation of unsaturated fatty acids, hydrogen abstraction from allylic methylenes produces allylic radicals in which electrons are delocalized through either three-carbon systems (oleate) or five-carbon systems (linoleate and linolenate). Reaction with oxygen at the end positions of these delocalized allylic systems produces well-defined mixtures of isomeric hydroperoxides. During autoxidation of oleate, four allylic hydroperoxides containing OOH groups on carbon 8,11 (*cis*-8-OOH, *cis*-11-OOH) and 9,10, (*trans*-9-OOH, *trans*-10-OOH) are produced. Autoxidation of linoleate yields formation of a mixture of *cis*, *trans* and *trans*, *trans* (9-OOH and 13-OOH) conjugated diene hydroperoxides [Frankel, 1998c]. Autoxidation of linolenate, in contrast to linoleate, forms initially significant amounts of secondary oxidation products together with hydroperoxides. As the number of double bonds increases in polyunsaturated fatty acids (PUFA), they produce more complex mixtures of hydroperoxides, which are easily decomposed and become very difficult to analyze quantitatively [Frankel, 1998c]. The relative rate of autoxidation of oleate: linoleate: linolenate was reported to be in the order of 1:40–50:100 on the basis of the oxygen uptake and 1:12:25 on the basis of peroxide development [Frankel, 1985, 1998a]. Termination can also occur by antioxidants that interrupt the free radical chain reaction.

Hydroperoxides, the primary oxidation products, are unstable and easily decompose involving monomolecular or bimolecular reactions [Belitz & Grosch, 1999]. Decomposition products – peroxy and alkoxyl radicals – are highly reactive and may act as initiators of autoxidation. Decomposition of hydroperoxides produces non-volatile monomeric compounds, including di- and tri-oxygenated esters derived from the corresponding keto-, hydroxy-, hydroperoxy- and epoxide esters [Frankel, 1985]. Monohydroxyperoxides of unsaturated fatty esters are also precursors of volatile decomposition products (pentane, heptane, octane, pentanal, hexanal, heptanal, octanal, decanal and others) [Gordon, 2001]. Unsaturated aldehydes and ketones undergo autoxidation, producing volatile compounds (dimers, oligomers, hydroperoxy epoxides hydroperoxy epidioxides and dihydroperoxides [Frankel 1998b]. Those secondary products decompose the same way as monohydroxyperoxides to produce similar volatile compounds.

Hydroperoxides formed at the initial stage of autoxidation are non-volatile, odourless and relatively unstable compounds. They decompose to form volatile aromatic compounds, which are perceived as off-flavours and as a warning that food is no longer edible [Gordon, 2001].

Another mechanism of oxidation occurs in the presence of sensitizer and UV-light. Photo-oxidation pathway is an alternative route leading to the formation of hydroperoxides instead of the free radical mechanism. Excitation of unsaturated fatty acid or oxygen may occur in the presence of light and a sensitizer. There are two types of photo-oxidation [Gordon, 2001]: I – an electron or a hydrogen atom transfers between an excited triplet sensitizer and a sub-

strate (PUFA), producing free radicals or radical ions; and II – triplet oxygen ($^3O_2$) can be excited by light to singlet oxygen ($^1O_2$), which reacts with the double bond of unsaturated fatty acids, producing an allylic hydroperoxide [Frankel, 1985]. This reaction results in a formation of a trans configuration. Products of oleate oxidation are 9- and 10-hydroperoxides, linoleate produces a mixture of 9- 10- (*trans*, *cis*), 12- 13- (*cis*, *trans*) isomers [Frankel, 1998d].

The third mechanism of oxidation is based on lipoxygenase activity. Lipoxygenase is an enzyme which is a very important source of hydroperoxides formed during oil extraction. Lipoxygenase produces similar flavour volatiles to those produced during autoxidation. A molecule of lipoxygenase contains an iron atom, which is in high spin state Fe (II) and must be oxidized to Fe (III) by fatty acid hydroperoxides or hydrogen peroxide. The active enzyme abstracts a hydrogen atom from the methylene group of a polyunsaturated fatty acid with the iron being reduced to Fe (II) [Gordon, 2001]. A conjugated diene system is formed, followed by reaction with oxygen. Peroxyl radical and finally hydroperoxide are generated. The second type of enzyme reacts with an esterified substrate, before the release of fatty acids by lipase, additionally ketodiene fatty acids are formed [Belitz & Grosch, 1999].

PROOXIDANTS AND ANTIOXIDANTS

There are many factors influencing the rate of fat oxidation. The phases present in food will also affect lipid oxidation by affecting the activities of antioxidants present [Gordon, 2001].

Oxidation of lipids is affected by a number of factors including: (1) processing and storage conditions (temperature, light, oxygen, metals, enzymes); (2) content of unsaturated fatty acids and their distribution in triacylglycerol molecule; and (3) the presence of antioxidants (inhibitors) or prooxidants (catalysts).

Prooxidants

Trace amounts of metals, *e.g.* Fe, Cu, act as promoters of lipid oxidation in the presence of hydroperoxides. Transition metal ions are involved in one-electron redox reaction, which leads to hydroperoxide decomposition [Bondet *et al.*, 2000]:

$$Me^{n+} + ROOH \rightarrow RO^{\bullet} + Me^{(n+1)+} + OH^-$$
$$Me^{(n+1)+} + ROOH \rightarrow ROO^{\bullet} + Me^{n+} + H^+$$

The alkoxyl and peroxyl radicals formed initiate the chain reaction of the autoxidation. A low concentration of ascorbic acid accelerates the decomposition of hydroperoxides as ascorbic acid reduces ferric ions. The lower oxidation state provides over ten-fold faster decomposition rate than the higher state [Belitz & Grosch, 1999]. Metals are thought to play a key role in the initiation of autoxidation process, as it is difficult to eliminate traces of metals during food processing. Metal ions may originate from plant and animal tissues. Influence of processing and storage conditions is also not negligible. Optimal pH for Fe and Cu activity is 5.5–6.

Free radicals, products of hydroperoxide decomposition, act as initiators of autoxidation. Thermal, metal-catalyzed or photo-catalyzed hydroperoxide decomposition results in the formation of hydroxyl-, alkoxyl- and peroxyl radicals, *e.g.* [Geoffroy *et al.*, 2000]:

$$ROOH \xrightarrow{\text{UV or Temp}} RO^{\bullet} + OH^{\bullet}$$

Radicals generated from food contaminants may also participate in oxidation process acting as catalysts.

Some molecules absorb UV light energy and are converted into an excited single state. In foods containing lipids photosensitized oxidation may be initiated by pigments, such as riboflavin and porphyrins (chlorophyll, hemoglobin, myoglobin) or some synthetic dyes [Frankel, 1998d].

Antioxidants

The substances able to inhibit or retard oxidation are referred to as antioxidants. They occur in foods as native constituents or as additives. Antioxidants can be classified according to the mechanism of action into two groups.

Primary antioxidants (chain-breaking antioxidants) are free radical acceptors. As they act as hydrogen donors they are able to scavenge lipid radicals, *e.g.*:

$$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet}$$

Antioxidant radicals are stable due to delocalization of the unpaired electron around a phenol ring and cannot easily react with fatty acids. They are able to terminate radical chain process by reacting with radicals, *e.g.* [Reische *et al.*, 2002]:

$$ROO^{\bullet} + A^{\bullet} \rightarrow ROOA$$

The most important primary antioxidants are: tocopherols, BHT, BHA, and PG.

Secondary antioxidants, in opposite to the primary antioxidants, do not break free radical chain but are able to act through various mechanisms, as: reducers and chelators of metals (*e.g.* citric acid, phosphoric acid, EDTA); oxygen scavengers and reducing agents (*e.g.* ascorbic acid, ascorbyl palmitate, sulfites); singlet oxygen quenchers (carotenoids) [Reische *et al.*, 2002]; and substances able to recover primary antioxidants (ascorbic acid).

The use of antioxidants as food additives enables protection of food lipids against undesirable oxidative changes. Synthetic antioxidants are relatively inexpensive, but their safety has been questioned. Consumers claim about the use of natural-origin food additives result in search for natural antioxidants, commonly perceived as safe, *e.g.* phenolic acids, flavonoids, tocopherols, ascorbic acid, carotenoids [Espin, 2000; Lu & Lu, 2001, Schwarz *et al.*, 2001; Małecka, 2002]. The potential sources of antioxidants are herbs, spices, fruits, vegetables, seeds, and tea. There is much concern about the use of natural extracts to protect lipid-containing foods. The effects of various plant extracts rich in polyphenols on the stabilization of lipids and model systems have been widely investigated [Abdalla & Roozen, 2001; Żegarska *et al.*, 1998; Astley, 2003; Loliger *et al.*, 1996; Shahdi & Wanasundara, 1992].

OXIDATION OF STEROLS AND FAT-SOLUBLE VITAMINS

Sterols and fat-soluble vitamins, as a part of an unsaponifable fraction of lipids, are readily susceptible to

oxidation by active oxygen and related species, as well as exogenous agents (UV, ionisation, sunlight, temperature, peroxides, radicals, metal ions, enzymes). Sterols and fat-soluble vitamins (named A, D, E and K) are all isoprenoic compounds synthesized by the condensation of multiple isoprenoid compounds [Moszczyński & Pyć, 1999].

Cholesterol (5α-cholesten-3β-ol) as the main animal sterol, and phytosterols represented by β-sitosterol (5-cholesten-24β-ethyl-3β-ol), campesterol (5-cholesten-24β--methyl-3β-ol) and stigmasterol (5,22-cholestadien-24--ethyl-3β-ol) can generate numerous oxidation products. More than 100 oxidation products of cholesterol (COP) have been identified until now, however only six to eight of these compounds are generally reported in foods, including: 7α-hydroxycholesterol, 7β-hydroxycholesterol, α-epoxycholesterol, β-epoxycholesterol, cholestane-triol, 7keto-cholesterol, 20α- and 25-hydroxycholesterol, and were detected in very high amounts in, for example, commercially-dried whole egg powder up to 200 ppm [Pie *et al.*, 1990], in powdered milk up to 30 ppm [Chan *et al.*, 1993], infant formulas [Przygoński *et al.*, 2000], and in fried pork loin up to 11 ppm [Echarte *et al.*, 2001]. Gordon and Magos [1983] proposed that the non-lipid radicals react rapidly with the unhindered allylic carbons of the sterols. COPs have been shown to have a wide variety of effects both *in vitro* and *in vivo*, which may be linked to human diseases and cholesterol metabolism.

Relatively little information exist on the formation of phytosterol oxidation products in food and their biological implications. Finocchiaro and Richardson [1983] assumed that oxyphytosterols are absorbed by humans and their subsequent metabolic conversions may be of toxicological significance. The toxic effects of oxyphytosterols on intestinal tissue should not be ignored, since 5,6-epoxy- and 7-ketosterols are potential alkylating agents, which might react with intestinal nucleophiles, thereby exerting toxic effect. The occurrence of oxyphytosterols in foods was much less studied than that of oxycholesterols. They were determined in rapeseed oil/palm oil blend, sunflower oil and high-oleic sunflower oil up to 41, 40 and 46 ppm, respectively [Dutta & Appelqvist, 1997; Dutta, 1997]. After two days of frying operations, these levels increased to 60, 57 and 56 ppm, respectively. The level of total sterol oxides in chips samples fried in sunflower oil was 46 ppm, and the lipid content in samples fried in high-oleic sunflower oil was 35 ppm. The content of oxyphytosterols in refined oils was 2 to 2.5-times higher than in cold-pressed oils from Polish markets [Rudzińska *et al.*, 2001]. Heating of refined plant oils (corn, rapeseed, sunflower and soybean) resulted in an increase of phytosterol oxidative derivatives concentration about 130% in rapeseed oil up to 485% in soybean oil [Rudzińska *et al.*, 2002]. The content of oxyphytosterols in rapeseeds was from 10 to 15 μg/g of seeds depending on variety [Rudzińska *et al.*, 2003].

Natural antioxidants such as raspberry, black currant, tomato seed, rosemary extracts and α-tocopherol exhibited the protective effect in peanut and purified sunflower oil [Małecka *et al.*, 2004; Rudzińska *et al.*, 2004]. Vitamin E keeps plant oils and sterols from forming lipid peroxides and oxidized sterols, acts as an antioxidant, and is consumed during the induction period of autoxidation. The tocopherols not only inhibit free radical-induced lipid autoxidation, but they also inhibit the oxidation induced by singlet oxygen. They react with singlet oxygen either by physical quenching or by chemical reactions. Oxidation of tocopherols generally gives similar products, like lactones, quinones, and other degradation products [Kamal-Eldin & Appelqvist, 1996]. The ability of tocopherols for oxidation determines their application as antioxidants added to vitamin A confections, lipids and other food products. Being highly unsaturated, vitamin A is susceptible to oxidation during processing and storage of foods. Isomerization of *trans*-carotenoids to *cis*-carotenoids, promoted by contact with acids, heat treatment and exposure to light, diminishes the vitamin A activity. The oxidation of carotenoids is not well understood. It involves initially epoxidation, formation of apocarotenoids and hydroxylation. Rising compounds of low molecular masses causes changes of colour and aroma of tea, wine, carrots, and tomatoes [Rodriguez-Amaya, 1999; Abushita *et al.*, 2000].

Not much data is devoted to oxidation of vitamins D and K. Both vitamins are light sensitive and the acidity or alkalinity of the environment can additionally activate their decomposition. Vitamin K is an essential nutrient for animals and humans because it is required for functioning of the blood clotting cascade. The antioxidant potential of vitamin K has been investigated by Talcott *et al.* [1985]. They showed that menadione is a very potent inhibitor, but active vitamins K with isoprenoid (vit. K_2) or phytyl (vit. K_1) chain at the C3 position weakened that activity. Vervoort *et al.* [1997] observed no antioxidant effect of vitamin K and vitamin K epoxides, which are formed during oxidation of vitamin K by hydrogen peroxide in alkaline environment. But the reduced form of vitamin K (K-hydroquinone) demonstrates antioxidant activity more potent than α-tocopherol [Mukai *et al.*, 1993]. Vitamin D is found in food, but also can be produced in human body after exposure to ultraviolet rays from the sun. It exists in several forms, each with a different activity. They are reliable for calcium and phosphorus absorption. Vitamin D promotes bone mineralization in concern with other vitamins and minerals, and hormones. In no lipids environment vitamins D are susceptible for oxidation agents and form 5,6-*trans*-vitamin D, which has only 10%–30% of vitamin activity. 5,6-Epoxide derivatives of vitamin D_2 are formed from vitamin D_2 in the presence of riboflavin under light [King & Min, 2002]. Vitamins D dissolved in plant oils are resistant at high temperatures and during long term storage. Vitamin D_2 is more stabile than vitamin D_3.

OXIDATION IMPACT ON SENSORY PROPERTIES OF FAT-CONTAINING FOOD

Product's quality has a very important influence on consumer's behaviour, particularly when it comes to selecting products to purchase and consume [Stone *et al.*, 1991]. Under the view of the consumer of foodstuffs, flavour by far holds the first place among the attributes participating to food quality. Consumption statistics of highly developed countries clearly indicate this fact [Rothe, 1988]. The flavour of various foods can undergo great changes during handling the raw material, processing into a product or during storage. Primary flavour compounds can be lost, secondary ones can be formed, sometimes to the benefit of the final product as for instance in maturation processes.

However, very often such changes lead to losses in sensory quality of food [Eriksson, 1978]. That is the case of fat and fat-containing foods, which undergo oxidation. Lipid oxidation results in off-flavours and odours indicating poor-quality products [Coppin & Pike, 2001]. Unsaturated fatty acids, which are extremely susceptible to the autoxidation, are prevalent in commercial oils, affecting their quality and shelf life.

Oxidation of fatty acids in food results in the formation of volatile compounds among which many have an unpleasant odour and are responsible for flavour problems in food industry [Grosch, 1982; Grosch *et al.*, 1992]. Since that, there is often a need to determine not only the present status of the oil but also its oxidative stability. Several tests have been designed to determine the induction period of oils but according to Coppin and Pike [2001], the onset of rancidity as determined by human sensory analysis is the ultimate test for calculating induction period. Thus "sensory induction period" can be defined as the time required for a fat or oil to become slightly rancid as determined by a sensory panel. The same authors obtained high statistical correlation between sensory scores and Oxidative Stability Instrument measurements done for soybean samples during light-exposed storage. The moderate linear relationship between "sensory induction period" (SIP) and Oxidative Stability Instrument (OSI) was obtained by Broadbent and Pike [2003] for stored canola oil.

As a result of fatty acids autoxidation, initially odorless monohydroperoxides are formed, which eventually break down into mainly volatile products. This group comprises aldehydes, ketones, alcohols, acids, hydrocarbons, furanones, and lactones [Grosch, 1982]. Due to the low odour threshold of the majority of these compounds, the presence of volatile hydroperoxide degradation products even at low concentration impairs the sensory properties of oils or fat-containing products. As the odour threshold of aliphatic aldehydes in oils can be as low as 0.0015 μg/L in the case of 2,6-*trans*, *cis* nonadienal, or 0.0001 μg/L for 1-octene-3-one [Grosch, 1982], sensory analysis can be regarded as a good tool for indicating oxidative changes of food quality. Vinyl ketone, 1,5-octadien-3-one and 2-nonenal after 42 days of storage increased 8-fold compared to the fresh sample and generated the unpleasant fatty off-odour in the stored butter fat [Grosch *et al.*, 1992]. The increase in the concentration of carbonyls in the presence of the pro-oxidant copper replaced the original odour of butter oil by fatty, tallowy, green off-odours. The odour activity value of carbonyls, particularly 2-nonenal, grew up significantly during storage of butter oil, as well as 1,5-octadien-3-one in boiled, frozen trout [Grosch *et al.*, 1994].

One of the important problems facing the meat and food industries is warmed-over flavour (WOF) in meats [Johnsen & Civille, 1986; Pikul, 1992a, b]. WOF is a sensory phenomenon and any analytical chemical works to determine causes and to find solution to this problem must be conducted in combination with sensory analysis of WOF. WOF often means despite cooked beef lean and fat also cardboard, rancid and fishy in terms of old fish smell, odours. Green, oxidized, rancid and cardboard odours are attributes more often used to describe sensory quality of stored fat-containing food.

Sensory profile of smell and taste has been developed in several studies of fat and fat-containing food quality estimation. A standardiszed descriptive language for sensory evaluating the flavour of pond-raised channel catfish has been developed [Johnsen *et al.*, 1987]. It contained terms of possible off-flavours; among them there were cardboard as aromatic associated with slightly oxidized fat and oils and reminiscent of wet cardboard. Fish oil can either be incorporated into food system after partial hydrogenation or with the fatty acids composition unaltered. Although improved deodorization processes have been developed, fishy off-flavours continue to limit the use of menhaden oil [Schnepf *et al.*, 1991]. Trimethylamine, as well as compounds from oxidizing polyunsaturated fatty acids, have been thought to be responsible for these off-flavours, which affect the sensory characteristics of products containing fish oil. Sensory odour profile methods was applied for the accelerated storage test of rapeseeds oils as well as for estimation of different plant oils stored at room temperature and good results of samples differentiation were obtained [Jeleń *et al.*, 2000]. In cases where the amount of compounds was highest, the samples were perceived as the worst (oxidized, green), whereas those with low levels of volatile compounds were the most desired ones according to sensory scores. PCA (principal component analysis) of chromatographic data of rapeseed oil stored at 60°C up to 10 days was related to PCA of sensory analysis and similarities in sample clustering were observed [Mildner-Szkudlarz, 2003].

According to Lawless [1991], the humane sense of smell is the ultimate discriminator of food aroma and flavour quality. Analysis of volatiles was very often ranked second after sensory analysis to evaluate the lipid oxidation in terms of their usefulness [Frankel, 1993]. Any chemical method used to evaluate shelf-live must be closely correlated to sensory analysis. Only sensory analysis can detect flavours due to oxidative and non-oxidative degradation processes [Broadbent & Pike, 2003].

HEALTH AND NUTRITIONAL IMPLICATIONS OF LIPID OXIDATION

Nutritional and toxicological effects of lipid oxidation in foods have attracted much interest recently [Frankel, 1996]. Possibilities of human exposition to oxidized fats in the diet from fatty fish and fish oils, deep fat frying, pre-cooked frozen and chilled foods and powdered foods have been considered [Kubow, 1990; Ziemlański *et al.*, 1991]. However, there is also a suggestion that in most cases oxidized fats are rendered unpalatable because of the deterioration in flavour and appearance long before the changes have appreciably reduced nutritive value or created toxicity [Gurr, 1988].

The most susceptible to oxidation are those oils which are rich in polyunsaturated fatty acids. Thus one nutritional effect of oxidation is to reduce the essential fatty acid content of edible fats. But the significance of that occurrence for nutrition is quite low since losses are usually small in relation to the total content of polyunsaturated fatty acids supplied by these susceptible oils. More serious nutritional problem of lipid oxidation is affected by interactions of lipid oxidation products with other food components, mainly with vitamins and proteins.

Several studies have demonstrated effects of feeding lipid oxidation products to experimental animals that may be interpreted as due to oxidative damage. There were

observed such symptoms of administration of oils and fats subjected to oxidation, as elevated liver and kidney weights, cellular damage in various organs, altered fatty acid composition of tissue lipids, cardiac fibrotic lesions, and hepatic bile duct lesions [Sanders, 1989; Addis & Warner, 1991; Kubow, 1990; Eder, 1999]. Studies on the possible pathological significance of lipid oxidation products were concerned on the effect of lipid peroxides, secondary products of lipid oxidation, especially malondialdehyde and cholesterol oxidation products [Addis, 1986].

The primary products of autoxidation – fatty acid peroxides - are probably not readily absorbed from the gut, and the most potent deleterious effects of lipid peroxides appear on the gastrointestinal mucosa. But it is discussed the probability, that oxidation *in vivo* and diet are both the sources of serum lipid peroxides [Addis, 1986]. Fatty acid peroxides have been shown to accelerate all three phases of atherosclerosis: initiation – endothelial injury, progression – accumulation of plaque, and termination – thrombosis [Kubow, 1990]. Dietary lipid peroxides participate in the development of cancer in humans. It was demonstrated a strong reaction between lipid peroxides and DNA [Addis, 1986].

Low molecular products of decomposition of fatty acid peroxides, however, are absorbed into the circulatory system and incorporated into the liver or have access to other body tissues. Malondialdehyde, a secondary product of lipid oxidation, has received much attention. Since it is a bifunctional aldehyde, it is a very reactive compound in cross-linking reactions with DNA and proteins [Addis, 1986; Kubow, 1990].

The toxicity of oxidized cholesterols has been demonstrated in several studies. The oxysterols are absorbed from the intestinal tract and are transported in the blood to arterial deposition sites at rates similar to cholesterol [Kubow, 1990]. There is considerable evidence that some cholesterol oxidation products are powerful atherogenic agents *in vivo* and *in vitro*. They have also cytotoxic and mutagenic properties [Addis & Warner, 1991; Osada *et al.*, 1998].

In summary, it must be realized that not a single product but a mixture of the above groups of lipid oxidation products can occur in daily diets. For this reason foods should be protected in any way to minimize their concentration in foods and eliminate their deleterious effects. Thus the use of natural antioxidants has been gaining considerable importance [Johnson, 2001; Virgili *et al.*, 2001].

ECONOMICAL ASPECTS OF USING ANTIOXIDANTS

Much attention is being paid to toxic effect of oxidized fats on structural and functional changes in many organs and tissues. Damage of biomolecules is associated with an increased risk of age-related diseases including cancer and cardiovascular heart disease (CHD) and may cause early death or disability, thus placing the financial burden on public health services. In Europe the mortality due to cardiovascular problems covers still more than 40% [Kromhaut, 2001].

Recent experimental and epidemiological data suggests that antioxidants, which are capable of preventing or retarding oxidation of lipids and other food constituents, are important factors in reducing the risk of chronic diseases. In the United States it has been calculated that compliance with nutritional guidelines concerning lipid consumption and in addition diet supplementation with high doses of antioxidant vitamins E and C would allow savings about 8.7 billion dollars a year in expenditure for medical care, because of the expected considerable drop in morbidity of cardiovascular heart disease and cancer [Tappel, 1995].

Antioxidants occur naturally in food, are intentionally added to products or formed during processing. They enable obtaining technological, economic and health effects whereas the most important ones are: extending the shelf life of food, maintaining unstable nutrient compounds and limiting disease risk connected with consumption of oxidized fats. Depending on the type of products there are various methods for evaluating these beneficial effects [Astley, 2003]. While for the investigators at assessment of antioxidants the most important is their activity in particular system, for the technologists an index of effectiveness, which expresses the relation of the effects obtained to the money spent, is always the most important one. The technological experiments carried out by Kwietniak and Harenza [1990] proved that one of the measurable effects of using antioxidants to fodder was mass increment of animals by 12–16% and also a decrease in fodder consumption per increment unit. The effects of stabilizing the fodder by using antioxidants are connected with the reduced losses of metabolic energy of fodder as a result of lipid oxidation. Prevention of lipid oxidation in fodder is therefore of essential benefit from the economic point of view. The decrease of nutrition effectiveness at implementing thermooxidised fats to a diet for experimental animals was also found [Wolfram, 1994]. Under some conditions, the addition of antioxidants can improve the quality traits of products. An intensive colour of egg yellow or of skin of poultry carcasses is a trait desired by a consumer and depends, to a great extent, on the content of carotenoids in fodder. Antioxidants, which protect carotenoids against oxidation, affect positively pigmentation of the above-mentioned products to meet thus the consumer's preferences.

Consumer attitudes towards food additives including antioxidants have to be considered by producers in creating purchase patterns taken up within marketing activities. Under equalization of demand and full availability of products on the market, consumers have a considerable influence on the quality of food products. At present, they prefer food of high sensory values, high nutritive value, and safe. They also expect meeting their needs at a reasonable price. There is also an increased interest noticed in natural products, for which consumer pays a higher price being assured that they contain neither contaminants nor chemical additives.

A synchronization of a trade offer on the market with real needs of consumers in the quantitative value as well as quality dimensions is a difficult task. It requires a penetrating analysis of information on the desired quality coming from the demand side of the market, connecting them with the data coming from a producer and a process engineer and taking up on this base a trial to create respective consumer needs and purchase patterns. A dislike of consumers to buy food containing synthetic chemical additives, among others antioxidants, preserving agents, and colorants, have been observed for a longer period of time. That phenome-

non is connected with a pro-ecological trend in various spheres of human activities in the last years and is a consequence of a fear of negative effects of excessive chemization of the everyday life.

At present, a consumer does not accept synthetic food additives. Because of that the work on introducing natural compounds which would extend food's shelf life, protecting the unstable nutritive components against unfavourable changes during storage, which would be safe simultaneously for a consumer, are answers to real needs coming from the market. One has still convince a consumer that the application of antioxidants and other functional food additives is not aimed at increasing producer's profits but is caused by objective factors, among which high quality and nutritive value of a product as well as health safety belong to the most important ones.

INTERACTION OF LIPID OXIDATION PRODUCTS WITH FOOD COMPONENTS

Lipid oxidation products are very reactive and can act with many food components to cause in a result many nutritional and sensory effects. Lipid peroxides decompose lipid soluble vitamins, such as vitamin E, A or its provitamins – carotenes, also water-soluble antioxidant vitamin C [Gurr, 1988]. As a result the overall dietary intake of these antioxidant vitamins is reduced, and the protective effect of these vitamins on the food itself is decreased. Peroxides and secondary lipid oxidation products, such as aldehydes and ketones, are able to react with vitamins of the B group and folate [Finley & Given, 1986; Ziemlański & Budzyńska-Topolowska, 1991].

The interaction of lipid oxidation products with proteins is also significant and there are several ways in which they react [Gurr, 1988; Finley & Given, 1986; Pokorny & Kołakowska, 2003]. Hydrophobic and hydrogen bonds between lipid peroxides or other products of lipid oxidation and protein are very extensive. But more important are reactions involving covalent bonds. Lipid free radicals can interact with several amino acids in protein molecules to induce protein free radicals. The end products of such reactions may be polymers formed by protein-protein cross-linking and complexes involving lipid-protein cross-links. The overall effect of these reactions is damage to amino acid residues, the most sensitive of which are histidine, cysteine/cystine, methionine, lysine, tyrosine, and tryptophan. Active oxygen species, which are generated during decomposition of lipid peroxides are also capable of reacting with these sensitive amino acids. Secondary lipid oxidation products (*e.g.* aldehydes, ketones and epoxides) are also capable of reacting with amino acids. Lysine is particularly susceptible to such degradation. Volatile fat oxidation products affect the flavour and safety of lipid-containing foods by causing damage to proteins and enzymes. The interaction of lipid hydroperoxides and secondary oxidation products with proteins and other components has also a significant impact on oxidative and flavour stability and texture during processing, cooking and storage [Frankel, 1998a]. All protein degradations will influence the nutritive value as well as protein functionality. These reactions lead also to the appearance of brown colour and production of unpleasant odours and taste [Pokorny & Davidek, 1979; Korczak *et al.*, 2000]. Colour of foods can be also altered by the oxidation of labile native pigments, like anthocyanins, carotenoids, chlorophyll or browning reaction products in baked foods by active peroxides formed during lipid oxidation [Finley & Given, 1986].

METHODS FOR THE ASSESSMENT OF LIPID OXIDATION

Methods for the quality assessment of lipids can be roughly classified into static ones, where a degree of oxidation is determined at a certain moment in time and dynamic methods in which fat or oil is subjected to accelerated aging process.

Methods for the assessment of lipid stability

The samples are subjected to accelerated stability tests to mimic changes that undergo in stored food. Usually, the induction period is measured as time after which a rapid change in rancidity or rate of oxidation occurs. In these tests, external factors are usually applied to accelerate the oxidation, *i.e.* elevated temperature, light, metal catalysts, elevated pressure, and air supply. The most popular ones are Schaal oven test, oxygen uptake, ASTM oxygen bomb, use of light or metal catalysts, Rancimat. Schaal oven test, which involves heating a sample to 60–70°C at atmospheric pressure is believed to correlate best the oxidative changes with evaluation of the shelf-life [Frankel, 1993]. The main flaws of high temperature stability tests are different mechanisms of oxidation, rapid decomposition of hydroperoxides, importance of side reactions – polymerization and cleavages, the rate of oxidation is dependent on oxygen concentration, losses of volatile antioxidants (BHA or BHT) can occur and very often the end point of measurement is questionable.

Methods for the measurement of lipid oxidation

There are several chemical and physical methods to assess the quality of fats and fat-containing foods. Peroxide value (PV) is probably the mostly used one in which concentration of peroxides (hydroperoxides) is determined as a measure of the extent of oxidation. Because of the unstable and intermediate nature of peroxides and their sensitivity to temperature, the PV is an approximate indicator of the state of oxidation but particularly in the early stage of oxidation it serves as a good tool for the measurement of a degree of oxidation. The iodometric determination of PV proposed by Lea is the most common [Gray, 1985]. The TBA (thiobarbituric acid) test is one of the most commonly used method and is based on the measurement of the absorbance of TBA-malonaldehyde complex at 532–535 nm. Malonaldehyde is a three carbon dialdehyde being one of intermediates formed in the oxidation of lipids. The objections to this method point out that depending on the aldehyde type peaks at different absorbance maxima are observed and TBA can react with other compounds, not being a part of the lipid oxidation system yielding also a red pigment. Total volatile carbonyl compounds – a measure which is related with off-flavour – can be also measured utilizing a formation of orange coloured hydrazones in the reaction of carbonyls with 2,4-dinitrophenylhydrazine and utilized as an indicator of oxidation process. Also anisidine

value is used for the assessment of a degree of oxidation, its conjunction with peroxide value (as Totox) and physical methods – measured conjugated dienes (at 234 nm) and trienes (at 268 nm) which can serve as a relative measurement of oxidation, or fluorescence based on the reaction compounds with a structure of N,N'-disubstituted 1-amino--3-iminoprene with peroxidising lipids [Gray, 1985; Angelo, 1996].

Chromatographic analyses of lipid oxidation products

Aldehydes, alcohols, ketones, furanones and lactones are the main volatile compounds generated in the lipid oxidation process. Aldehydes, especially unsaturated ones and acids produced during their oxidation are the main compounds, responsible for characteristic rancid off-odour of oxidized fats, hexanal, pentanal, hexanal, octanal, 1-octene--3-ol are often cited in the literature as characteristic compounds of plant oils oxidative degradation [Snyder *et al.*, 1985]. The correlation between rancid off flavour and contents of volatiles such as 2-pentenal, hexanal, 2-heptenal, octanal and nonanal has been reported [Solinas *et al.*, 1987; Angelo, 1996]. Therefore chromatographic methods allow monitoring compounds that have influence on the sensory properties of fats or fat-containing foods.

For the isolation of volatile products of lipid oxidation static headspace [Medina *et al.*, 1999; Boyd *et al.*, 1998], as well as dynamic headspace methods are used [Hartvigsen *et al.*, 2000; Morales *et al.*, 1994], the latter one especially in cases where high sensitivity is needed for monitoring the amounts of compounds present in ppb levels. Apart from these methods, solid phase microextraction (SPME) is gaining popularity in lipid oxidation volatiles analysis due to the robustness, simplicity, low cost and sensitivity comparable to purge and trap methods [Doleschall *et al.*, 2001, 2002; Jeleń *et al.*, 2000; Marsili, 2000].

The chromatographic methods developed in the 1970--ties for monitoring volatile oxidation product involved novel solutions: direct gas chromatography (DGC) [Dupuy *et al.*, 1971] – which involved placing up to 1 g of oil into a liner of GC and subsequent heating it to vaporize volatiles present in the analysed oil, and external closed inlet device (ECID) developed by Legendre *et al.* [1979]. The latter could be incorporated into any GC and was a combination of inlet assembly, a condenser and a six-port rotary valve and could work as a purge and trap device. A variation of DGC referred as direct thermal analysis (DTA) or short path thermal desorption has been developed as a result of earlier experiments [Manura & Hartmann, 1992]. The applications of chromatographic methods for the determination of volatile compounds in such commodities as peanuts, rice, beef, lamb, poultry, and fish have been extensively reviewed by Angello [1996]. Chromatographic methods are used not only for the analysis of volatile compounds in oils and fat-containing foods and resulting from the autoxidation process but also for the analysis of other minor constituents in oils [Cert *et al.*, 2000]

Chemometrical methods and electronic noses

Because of the vast amount of data generated in analysis of lipids oxidation compounds, chemometrical methods are used, usually principal component analysis (PCA), cluster analysis (CA) or partial least squares (PLS). Statistical methods are utilized for the processing of chromatographic data, or used in novel approaches to the analysis of volatiles, such as in machine olfaction.

The concept of electronic nose developed in early eighties within the last few years has gained popularity due to improvements in sensor technologies and software. Defined as an array of electrochemical sensors of diverse specificity, equipped with an identification tool capable of differentiating simple or complex flavours [Gardner & Bartlett, 1994] is a very promising tool also in the quality assessment of oils and fat-containing foods.

Aparicio *et al.* [2000] used successfully gas chromatography and an electronic nose based on conducting polymers for the identification of rancid smell in olive oil. Shen *et al.* [2001] showed the applicability of an electronic nose in monitoring flavour changes during accelerated storage of soy and corn oil, and obtained good correlations between PV value, sensory profiling and sensor signals. The electronic nose enabled not only monitoring the quality of oils but their geographical origin. Also a combination of SPME with mass spectrometry of unresolved mixture of volatiles, with statistical treatment of such "mean" spectra, found application in the investigation into changes in the shelf life of fat-containing products such as milk [Marsili, 1999, 2000].

CONLCUSIONS

It is believed that for the determination of oxidative stability, shelf life and consumer acceptance of products, methods for the determination of lipid oxidation can be ranked in the following order: sensory analysis > headspace analysis of volatiles > oxygen absorption > Peroxide Value > Thiobarbituric acid reactive substances (TBARS) > carotene bleaching by cooxidation with linoleic acid > Rancimat test [Frankel, 1993]. The diversity and abundance of methods used to monitor lipid oxidation reflect the complexity of this issue and confirm the fact that multiple methods should be applied to get the maximum information available.

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FINAL REPORT

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THE METHODOLOGICAL BASES OF THE EVALUATION OF THE QUALITY AND SAFETY OF THE NEW GENERATION FOOD (PBZ-KBN-020/P06/1999).

Title of the individual project:
The application of chemometric methods for quality evaluation of food enriched with natural antioxidants.

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Lipid oxidation, natural antioxidants, oxysterols, plant oils, lard, peanut, meat balls, fast gas chromatography, principal component analysis, SPME, MOS electronic nose, chemometric, GC/MS, lysine, methionine, green tea extract, rosemary extract, black currant seed extract.

SYNTHESIS OF RESULTS

For the food supplementation several natural antioxidants formulae were used, both commercially available and obtained in laboratory: rosemary extracts, black currant seeds extracts and green tea extracts. Their antioxidative activity was compared with BHT and tocopherols.

The antioxidative properties were determined in fatty substrates with a different degree of saturation and various physical constitutions. For this research the following substrates were used: fatty acids, their methyl esters, triacylglycerols (trilinolate and trioleinate) triacylglycerols of plant oils (sunflower, rapeseed and soybean) and refined oils and edible fats (sunflower oil, rapeseed oil, soy oil and lard). The fatty substrates were investigated in one-phase model and as water emulsions.

Stability of the model systems was evaluated using instrumental methods (Rancimat and Oxydograph) as well as classic Schaal test. Results were confirmed in systems mimicking food products, in which triacylglycerols of sunflower oil were used either in emulsion, in their natural form, and distributed on a solid matrix (cellulose).

As natural systems for the evaluation of antioxidants activity meat products after heat treatment and subsequently stored frozen were used. Pork and mackerel meatballs were used for this purpose.

Degree of oxidative changes in samples was evaluated based on the primary oxidation products (conjugated dienes, peroxide value) and the secondary products of lipid oxidation (anisidin value, TBARS and hexanal).

As the first step, oxidation indicators of the model system, consisting of methyl linolate emulsion, with or without the addition of BHT and incubated in the presence of Fe^{+2} as pro-oxidant were investigated. A degree of oxidation was determined using traditional indicators and volatile compounds. Traditional indicators confirmed a typical oxidation pattern with its acceleration in the presence of Fe^{+2} ions and retardation when BHT was added. However, mutual proportions of particular volatile compounds did not follow traditional indicators of oxidation.

In investigated rapeseed and sunflower oils emulsions high activity of natural antioxidants (rosemary extract, green tea extract, black currant seeds) was observed. It was lower than that of BHT, but comparable to α-tocopherol activity. The content of peroxides and linoleic acid, conjugated dienes indicated their formation by rosemary extract and black currant seeds, in a degree comparable to α-tocopherol, however to a lesser degree than with BHT. In rapeseed oil emulsions antioxidative activity presented the following (decreasing) order: rosemary extract OS > rosemary extract WS = black currant seeds extract. In the emulsion of triacylglycerols of sunflower oil the order was following: BHT > > green tea extract = rosemary extract > α-tocopherol.

The activity of antioxidants was different and related to the degree of fatty acids saturation and the environment. The antioxidants had different abilities in preventing primary and secondary oxidation products formation. It was shown that substrates of higher level of unsaturated fatty acids are more stable after the addition of antioxidant than stabilized substrates especially those based on more saturated fatty acids. Green tea extracts exhibited weaker antioxidant properties in emulsions of fatty acids of different degrees of saturation than BHT, and other examined natural antioxidants.

Ethanolic extract of green tea often had antioxidant properties superior to BHT α-tocopherol and rosemary extract. Lower activity in examined systems was observed for water extracts of green tea.

The addition of ethanolic extracts of green tea significantly improved the stability of pure TAGs and TAGs on a cellulose matrix. In investigated emulsified TAGs the ability of green extract to stabilize lipids was much lower than that of BHT.

A high antioxidant ability of ethanolic extracts of green tea was utilized for the lipids stabilization in chosen natural products, and their subsequent analysis by chemometrical methods.

Explored in this project a hyphenation of solid phase microextraction (SPME), fast gas chromatography and analysis of data using PCA for the analysis of volatile compounds of plant oils proved its suitability for oils differentiation based on the profile of volatile compounds. The differentiation was possible for oils of different source (plant), and a differentiation of fresh oils from that subjected to accelerated storage tests. Similar profile of volatile compounds was observed for fresh rapeseed and soy oil, and also for peanut and sunflower oils. Olive oil with a totally different volatile compounds profile was also characterized with the highest Totox value. Fresh analyzed oils, except olive oil, contained low amounts of volatile compounds that increased during storage. A significant correlation 0.95 ($p<0.1$) between Totox value and volatile compounds extracted using SPME was observed for both fresh oils and oils subjected to storage. Oils differentiation based on the instrumental method of volatiles measurement was similar to results of sensory profile analysis using PCA.

The suitability of metal oxide sensors (MOS) type electronic nose to the monitoring of fatty acids oxidation products was also investigated. The method was developed for the analysis of fat-containing matrices. Method parameters were elaborated based on a model system of rancid rapeseed oil containing the following 13 compounds, characteristic for oxidized rapeseed oil: hexanal, nonanal, E-2-pentenal, 1-octene-3-ol, octanal, E-2-octenal, pentanal, heptanal, 1-pentanol, E, E-2,4-decadienal, E-2-heksenal, E-2-dekenal, and E-2-nonenal.

In the first experiment, the concentration of compounds in the oil was 1, 2, 3, 4, 5 ppm (as for hexanal), in the second experiment lower concentrations (0,2; 0,4; 0,6; 0,8; 1 ppm) were investigated. Volatile compounds were isolated by SPME or static headspace (for MOS electronic nose). Various extraction times (7, 15, 30 min) and temperatures (35, 40, 50°C) were tested. Optimal parameters were chosen based on the lowest CV for samples in a cluster and the distances between clusters. For chosen parameters all the CV values were < 10%.

For the MOS electronic nose samples from the first series (higher concentrations) were easily differentiated (30-min incubation at 35°C), with a discrimination index of 90%. However a proper differentiation of samples within the low concentration region 0–1 ppm was impossible with this instrument, probably due to the relative low sensitivity of static headspace sampling.

To compare the performance of electronic nose with sensory panel results samples differentiation based on triangle test was performed. Minimal number of uniform answers for the determination of differences significance in triangle test equals 35 for an 80 people panel ($\alpha=0.05$). Only 28 people were able to properly distinguish samples, therefore electronic noses ability for samples differentiation was higher than that of sensory panel. Hyphenation of SPME directly with MS and SPME-fast GC-PCA facilitated the differentiation of samples in low concentration impossible to achieve using MOS e-nose.

Developed methods were utilized for the fast determination of oils quality, and the attempt to correlate chemometric methods with traditional indicators of oils oxidation was made. Five fresh oils (rapeseed, soy, sunflower, peanut, olive oil) were compared to oils subjected to accelerated storage tests. Chemometrical methods enabled differentiation of analyzed oils. Differentiation clusters pattern was similar to that of sensory profile analysis. Significant correlation 0.96 and 0.87 ($p<0.01$) between the results obtained using volatiles analysis by SPME-fast GC-PCA and SPME-MS and Totox value were observed. Significant correlation was also noted (0.84; $p<0.01$) between volatile compounds determined using GC-FID and GC-MS for both fresh and stored oils.

Samples of rapeseed oils stored under different conditions were also investigated. All examined chemometrical methods enabled differentiation of examined oils. Significant correlation (0.91; $p<0.01$ and 0.81; $p<0.05$) between volatile compounds determined by SPME-fast GC and SPME-MS and the Totox was observed. High correlation (0.73; $p<0.05$) between GC/FID and GC/MS was observed.

Oils stabilized with the antioxidants were investigated using elaborated methods. It was observed that control oil samples (stored without the addition of antioxidants) were radically different from samples with added antioxidants. Oils stabilized with black currant pips were located closest to non-stored fresh oil samples, which suggests their good quality.

Effect of antioxidants addition (BHT, α-tocopherol, rosemary extracts and green tea extracts) on the TAG fraction of the sunflower oil was investigated. MOS based e-nose was able to distinguish samples with the addition of antioxidants from control samples (without antioxidants added). Ethanol extracts of green tea and rosemary extract showed antioxidant properties similar to these of BHT.

Volatile compounds of frozen meat products were investigated using chemometrical methods. Similar effectiveness of added antioxidants was observed. Samples to which rosemary extract was added differed in the profile of volatiles at the beginning of storage from remaining samples. At the end of storage they were located near BHT added samples. Samples to which green tea extract was added were similar to controls. Similar grouping was observed using MOS e-nose system. After 6 months of storage, a low degree of oxidation of rosemary added sample located it close to BHT added one, despite the detectable odor of rosemary. Similar results were obtained using sensory profile method, where the highest intensity of oxidized flavor were observed in control sample, slightly lower in samples with green tea extract and the lowest in samples to which rosemary extract and BHT was added.

High activity of plant extracts was confirmed when TAGs from sunflower oils were analyzed on a cellulose matrix using traditional indicators of lipids stability (peroxide value, anisidin value, Totox) and the sensory analysis and e-nose assessment. The addition of ethanolic extracts of green tea and rosemary had comparable antioxidation activity to that of BHT. Sensory analysis supported chemical analyses.

Pork meatballs were used to determine the influence of plant extracts addition (rosemary and green tea) on the oxidative stability of frozen meat products, which underwent thermal treatment prior to freezing. Green tea extract did not retard oxidation of lipids measured using peroxide

value, anisidin value and Totox as much as rosemary extract and BHT did. When TBARS was used as meat oxidation indicator, the effectiveness of green tea extract was the highest over the whole storage period. Both green tea extract and rosemary extract were more active than BHT.

An important indicator of oxidation changes in lipids, from the nutritional point of view, is the content of essential amino acids. It has been shown that protection of fats from oxidation process by antioxidant addition stabilized selected amino acids in meat and fish products and in model systems. An analysis of amino acids stability showed that oxidative changes of lipids caused a decrease in lysine and methionine levels, but it was restricted by antioxidants addition. Extract of green tea was more active than that from rosemary. Natural antioxidants, however, were less effective than BHT during a 6-month storage period. Samples with the addition of BHT had 83% more lysine than the control, whereas in samples with the addition of green tea extract and rosemary the values were 63% and 53%, respectively.

During storage experiment higher losses of methionine than lysine were observed (66% to 77% compared to initial values). The addition of antioxidants restricted the losses. The most effective was rosemary extract – samples with its addition contained 50% more methionine than controls, whereas in samples to which green tea extract was added only 16% more methionine was observed. In samples with BHT addition 41% more methionine was observed compared to control.

The addition of antioxidants influenced also the digestibility of meat products proteins. Directly after thermal treatment (cooking) higher *in vitro* protein digestibility was observed in samples with antioxidants addition (80% compared to 76% in control). After six-month storage the differences increased and the highest digestibility was observed in samples with green tea extract.

In frozen fish products the addition of antioxidants inhibited the decrease in lysine and methionine content.

It favoured also the higher digestibility of protein. The improvement of protein digestibility and minimizing essential amino acids losses were correlated to the restriction of oxidation processes in lipids, determined with traditional methods. Also in model systems (incubated emulsions of fatty acids ethyl esters), the most significant losses were observed in samples to which no antioxidants were added, or the pro-oxidant was added. The highest decrease in lysine and methionine contents were observed in polyunsaturated fatty acids emulsions. The differences were less pronounced in monounsaturated acid emulsions and almost non-existent in saturated acids emulsions. The losses of essential amino acids were correlated to the degree of oxidation of incubated fat-containing extracts.

The presence of oxysterols is one of the indicators of food quality related to oxidation of cholesterol or phytosterols. The impact of natural antioxidants addition on the sterols stability was also investigated. As the first step, the products of phytosterols oxidation in model systems were determined. The model systems included stigmasterol and β-sitosterol solutions in trioleate, which were heated in Oxidograph under different temperature conditions. In samples peroxide value and anisidin value, fatty acids composition, phytosterols content and oxyphytosterols content were determined.

β-sitosterol solution contained 63% of this compound and 34% of campesterol. Stigmasterol solution contained 88% of this compound (compared to total sterols). The content of β-sitosterol and campesterol in heated model system did not undergo statistically significant changes. The content of stigmasterol in model system equaled to 18.7 mg/g. In model sterols solution six oxidation products were determined – 7α- and 7β-hydroxy-, α- and β-epoxy-, triol- and 7-keto- derivatives of campesterol, β-sitosterol and stigmasterol. It was observed that in the unheated β-sitosterol solution in trioleate the total content of oxyphytosterols was 18.96 μg/g, including 6.02 μg/g of campesterol derivatives and 12.94 μg/g oxidation products of β-sitosterol. In the solution of stigmasterol the content of its oxidation products was 18.18 μg/g. In the course of heating of investigated products significant increase in oxidation derivatives was observed – from 44.66 μg/g to 155.63 μg/g in sitosterol solutions and from 43.48 μg/g to 171.87 μg/g in stigmasterol solution, depending of the applied heating conditions.

The addition of ethanolic extracts of green tea, rosemary and BHT and α-tocopherol to the stigmasterol solution in TAG of sunflower oil and β-sitosterol solution in TAG of sunflower oil significantly inhibited the degradation of analyzed phytosterols. In the explored model system the effectiveness of investigated antioxidants in the inhibition of phytosterols degradation was as follows: BHT > green tea extract > α-tocopherol > rosemary extract. Formation of stigmasterol oxidation products was the lowest in the sample with α-tocopherol and ethanolic extract of rosemary. In the all analyzed samples, α-epoxy-stigmasterol was formed in the highest amounts among the analyzed stigmasterol oxidation products. Similar results were determined for the content of β-sitosterol oxidation products. Ethanolic extract of rosemary and α-tocopherol had higher inhibition activity. The content of oxyphytosterols was lower by about 20% than in control sample. In these samples β-epoxy-sitosterol dominated among the analyzed sitosterol oxidation products.

Treatment of peanuts with rapeseed oil enriched with antioxidant seed extracts was used to prevent phytosterols oxidation. Ethanolic extracts of raspberry, black currant and tomato seeds obtained from the waste of food processing were used as potential antioxidants. All extracts exhibited the protective effect towards fatty acids as well sterols oxidation in peanut samples although the black currant seeds extract was the most effective one.

The content of cholesterol and its oxidation products 7α- and 7β-hydroxy, α- and β-epoxy, triol, 20α-hydroxy-, 25-hydroxy- and 7-ketocholesterol were determined in pork meat balls every 60 days of storage at -20°C. The total increase of the cholesterol oxidation products was the lowest in all samples with ethanolic extract of rosemary. The ethanolic extract of green tea also showed antioxidative properties, but the content of oxysterols in control samples and in samples with addition BHT did not show statistical significantly differences. In all analyzed samples, 7α--hydroxy-cholesterol was formed in the highest amounts among the analyzed cholesterol oxidation products.