Oxidative fate of lipid structures in food and during digestion – possible metabolic significance

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Abstract. *Although excessive lipid consumption should be avoided, dietary lipids are now recognized as having various beneficial nutritional effects, especially regarding the need for a balanced supply in both omega-6 and omega-3 polyunsaturated fatty acids (PUFA). Improving the lipid profiles through an increase of PUFA supply also increases risks of (per)oxidation with possible negative consequences for food technological, sensory and nutritional properties. Food lipids exhibit various molecular and supra-molecular structures that interact with the other constituents of the foods. These structures and interactions evolve during processing, storage and digestion. They modulate or induce the formation in the food and in the digestive tract of potentially hazardous molecules. This short review concerns the links between the structures of lipids in foods, their oxidation and the metabolic fate and significance of lipid oxidation products. The actual data concerning: (ii) oxidation of dietary lipids, especially when dispersed as oil-in-water emulsions, during process including digestion, (iii) the absorption of primary and secondary, lipid oxidation (especially hydroperoxides and the two hydroxyalkenals : 4-hydroxy-2-nonenal and 4-hydroxy-2-hexenal) and their consequences on metabolic oxidative stress are briefly summarized. We conclude that innovative food formulations should integrate an adequate structuration of lipids so that their metabolism could be oriented toward their nutritional benefits, limiting the risks linked to peroxidation of the unsaturated fatty acids in the food and during digestion.*

Keywords. Dietary lipids, omega-3 supply, food, oxidation, digestion, metabolic fate, hydroperoxides, hydroxyalkénals.

Introduction

Over the past 30 years, omega-3 (n-3) polyunsaturated fatty acids (PUFA), and among them the long-chain PUFA, eicosapentaenoic acid (EPA : C20:5 n-3) and docosahexahenoic acid (DHA : C22:6 n-3), are recognized to be of major importance in human nutrition and disease prevention. Disorders of human health linked to an imbalanced overproduction of eicosanoids derived from omega-6 (n-6) polyunsaturated fatty acids could be, at least in part, counterbalanced by a significant increase of the n-3 PUFA dietary intake. A balanced, n-6/n-3 FA ratio (around 5 according to the recommendations) in the diet may reduce the risk of various life-style diseases. In France, recent daily recommendations for healthy adults account for about 2 g linolenic acid (ALA : C18:3 n-3) and 0.5 g EPA + DHA but in several specific metabolic or pathologic cases higher intake (up to several g EPA + DHA) is proposed. The different ways to re-equilibrate the omega-3 PUFA human intake should be accordingly explored taking into account the actual ways of life, wishes and economic status of the specific populations. One way is the enrichment of formulated foods. The number of omega-3 enriched foods proposed to the consumers has accordingly dramatically increased these last years. It remains that the acceptability and shelf life of these new products is often low due to the frequent, rapid and often unpredictable development of off-flavours, including burp off-flavours, attributed to the formation of lipid oxidation odorant compounds.

The rancid and unpleasant taste/smell of oxidized omega-3 lipids usually prevents the intake of large amounts of lipid oxidation products. It is therefore currently believed that oxidation does not represent a health risk because the food would be rejected before consumption due to unacceptable sensory properties (Dobarganes & Marques-Ruiz, 2003). However it remains unresolved whether chronic intake of small amounts of peroxidation products produced either in the food during its industrial or home processing or during digestion presents a health hazard.

Lipid oxidation and formation of lipid oxidation products in foods during their processing, storage and digestion depend on various parameters, including the molecular structure of the oxidizable species and their organisation in the matrix, in interaction with the physico-chemical and environmental conditions that evolve with time, especially in the digestive conditions.

This short paper intends to summarize the actual knowledge and questioning about the links between the structures of lipids in foods, their oxidation before and after ingestion and the metabolic fate and significance of lipid oxidation products.

Oxidation of dietary lipids during food processing and storage

The mechanisms of lipid oxidation reaction has been extensively studied even is some reaction pathways and kinetic aspects of the reaction are not completely elucidated (Frankel 2005) The autoxidation of lipids is described as an autocatalytic chain reaction that targets unsaturated fatty acids and involves free radicals intermediates. The main primary products of autoxidation, the hydroperoxides are odorless and tasteless. Secondary oxidation products issued from decomposition of hydroperoxides comprises an array of odorant volatile compounds including aldehydes, ketones, alcohols, hydrocarbons, ... responsible for the development of off-flavours . Other secondary products of low molecular weight, including malondialdehyde (MDA), hydroxy-alkenals : 4-hydroxy-2(E)-nonenal (4-HNE), 4-hydroxy-2(E)-hexenal (4-HHE) are recognized markers of oxidative stress *in vivo* where they exert cytotoxic activities. In addition, the decomposition of lipid hydroperoxydes leads to the formation of other compounds of higher molecular weights in which the glyceride backbone is kept.

Lipid oxidation products as influenced by the molecular structure of lipids

Due to the high number of lipid molecular species containing unsaturated fatty acids in natural lipids, and the multiple possible reaction pathways per fatty acid, the development of lipid oxidation leads to the formation of a very large array of oxidation products. These products vary in their molecular weight, structure, characteristic chemical function, physico-chemical and biological properties, including their odour. The positioning of the double bonds on the fatty chain determines the formation of low molecular weight products among them some are characteristic of the fatty acid series; for example, 4-HNE and hexanal for n-6 PUFA, 4-HHE and propanal for n-3 PUFA. By contrast MDA originates from both series. The molecular structure of the lipids, i.e. free fatty acids *versus* ethyl esters and triacylglycerols or sn-1 or sn-2 location of the unsaturated FA on the glycerol backbone also influences the oxidation rates.

Factors that influence lipid oxidation in food formulations.

Most of food formulations contain lipids as a hydrophobic phase dispersed in a more or less hydrated hydrophilic phase. Indeed the presence of antioxidants is a main factor for the development of oxidation. The physico-chemical characteristics of the interfaces (Berton, Ropers, Viau & Genot, submitted) and of the aqueous phase (Villière, Viau, Bronnec, Moreau & Genot, 2005) also greatly influence lipid oxidation. Indeed, the dispersion states, the local environment of the fatty acid chains, either in the hydrophobic core of the fat droplets or at the interfaces and their interactions with antioxidants and pro-oxidants are other important factors (Genot, Meynier & Riaublanc, 2003 ; Waraho, Mc Clements & Decker, 2011).

Oxidative fate of lipids during digestion

When ingested as bulk oils or dispersed in solid or liquid foods, dietary fats are submitted to mechanical, chemical and biochemical process that make the fatty acids bioaccessible through the hydrolysis of the lipid molecules in the gastrointestinal tract (GIT). In fact, some conditions of the digestive environment could favour the formation of lipid oxidation products.

In-mouth : a primary reorganisation due to mastication and saliva

In the mouth, the ingested food is mixed and diluted with saliva. The pH, the ionic strength and the temperature change. Destructuration of the food matrices and/or dispersion of bulk fat can be observed. Food and saliva constituents are put into contact in the shear conditions induced by mastication. Mucin induces the flocculation of lipid droplets of food emulsions (Vingerhoeds, Blijdenstein, Zoet & van Aken, 2005) whereas some antioxidants (nitrite and thiocyanate) and/or pro-oxidant constituents (lactoperoxidase) of the saliva could exert some activity in the stomach (Gorelik, Kohen, Ligumsky & Kanner, 2007).

The stomach: a bio-reactor that may favour lipid oxidation

In stomach, the bolus, composed of the chewed food products ingested during the meal, is mixed with the gastric juice (pH 1-3). The lipids of the bolus come into contact with endogenous surfactants that leads to great structural reorganisations. The bulk fat is coarsly emulsified whereas small droplets would partially coalesce under the joint actions of the flocculation induced by mucin, the pepsinolytic activity and the acid pH (Armand, Pasquier, Andre, Borel, Senft, Peyrot, Salducci *et al.*, 1999). Partial lipolysis by gastric lipase also occurs. Lipolytic and pepsinolytic activities generate new surfactant molecules (fatty acids, mono and diglycerides, peptides) which in turn promote emulsification. The increase in exchange surface between lipids and gastric environment, the acidic conditions, the presence of oxygen and pro-oxidant factors

such as dietary iron or heme proteins (myoglobin) from meat products may promote lipid oxidation. Therefore, according to (Kanner & Lapidot, 2001), the stomach can be regarded as a bioreactor favourable to lipid oxidation. In the physico-chemical conditions of the stomach, the reaction is strongly influenced by the nature of the interfacial layer of dietary lipid droplets (Lorrain, Dangles, Genot & Dufour, 2010).

Duodenum: release and absorption of nutrients and oxidized species

The partially hydrolyzed and emulsified lipids are delivered into the small intestine where are mixed with pancreatico-biliary and intestinal juices. These juices contain bile salts, phospholipids, pancreatic lipase, colipase, proteases, ion minerals, bicarbonate, among others. It results an alkalinization of the chyme and additional modifications of the lipid interface and lipid structures. Lipolysis, proteolysis and amylolysis are completed. Mixed micelles and vesicles are formed allowing the absorption of fatty acids and monoglycerides. Unlike the prooxidant gastric environment, the intestinal environment is neutral or slightly basic, anoxic and of reducing nature. In addition, pancreaticobiliary juice would contain free radical scavengers although other compounds could induce the decomposition of the primary products of lipid oxidation (Terao, Ingemansson, Ioku, Yuki & Ito, 1995). Thus, in the small intestine, only secondary step of lipid oxidation could occur. The already present oxidized fatty chains linked to triacylglycerol molecules are hydrolyzed by lipases (Wilson, Lyall, Smyth, Fernie & Riemersma, 2002), making the oxidized moiety available for subsequent absorption. Both primary (hydroperoxides) and secondary (hydroxylated FA, hydroxy-alkenals, oxygenated α,β-unsaturated aldehydes: Oα,βUA) products can be absorbed in the intestine (Goicoechea, Brandon, Blokland. & Guillén, 2011). Gastrointestinal glutathione peroxidase has been proposed as a barrier against the absorption of dietary hydroperoxides (Wingler, Müller, Schmehl, Florian & Brigelius-Flohé, 2000).

Metabolic / health impact of oxidation of dietary lipids

It should be first underlined that if omega-3 PUFA enriched foods are beneficial for the prevention of oxidative stress and inflammation processes, a too high consumption of PUFA n-3 can exert pro-oxidants effects.

From a nutritional point of view, consumption of oxidized lipids in foods is often considered not representing a health risk: the food is assumed to be rejected due to unacceptable sensory properties before a significant decrease of PUFA and other oxidation sensitive nutrients could be registered. However unavoidable small amounts oxidized lipid formed during storage or after ingestion are generally ignored. However, lipid oxidation products may have many biochemical effects (Guéraud, Atalay, Bresgen, Cipak, Eckl, Huc et al, 2010)

Hydroperoxides: absorption and possible metabolic impact

Hydroperoxides are highly toxic compounds when administered intravenously, but in the body, apart from pathological situations, reactive compounds and hydroperoxides produced *in vivo* are detoxified by enzymatic cycles involving glutathione peroxidase. Early work suggested that dietary hydroperoxides does not cross the intestinal barrier and are eliminated in feces. However, the administration to animals, mostly rats, of diets containing oxidized oils caused the development of various pathologies such as cell injuries in different organs, increase of the weight of kidney and liver, modification of the fatty acid composition of adipose tissues, or changes in levels of prostaglandins suggesting an inflammatory response. The administration of vitamin E, antioxidant compounds may in some cases, prevent from these effects. More serious pathologies such as severe irritation of the gastrointestinal tract, diarrhea, and growth delays,

even the death of animals, have been noticed with animals fed with high amounts of highly oxidized oils, but the studies suffered of several experimental bias. More recent studies have demonstrated that peroxides compounds can accumulate in the intestinal lumen and contribute to the development of chronic intestinal disorders or diseases such as cancers (Kanazawa, Sawa, Akaike & Maeda 2002).

Secondary lipid oxidation products

In adult, consumption of diet containing triglycerides including hydroxylated oxidized PUFA led to the presence of these altered fatty acids in chylomicrons within hours after the meal (Wilson, Lyall, Smyth, Fernie & Riemersma, 2002). Alkenals such as 4-HHE and 4-HNE are strongly electrophiles reagents which can easily react with neutrophil groups of proteins, nucleic acids, lipids or with thiols of low molecular weight (e.g.: glutathione). When an alkenal reacts with an enzyme, it can alter its biological function. Thus, 4-HHE and 4-HNE are likely to exert deleterious biological effects, those induced by 4-HNE being the most documented. When aldehydes generated from the oxidation of linoleic acid were eaten by rats, oxidation products including 4-HNE were proved to be slowly absorbed by the intestine and accumulated in the liver (Kanazawa & Ashida 1998). The very reactive oxygenated α,β-unsaturated aldehydes (Oα,βUA) were also found to be bioaccessible (Goicoechea, Brandon, Blokland & Guillén 2011). 4-HNE exert pro-oxidant effect in vitro, suggesting that it could contribute in vivo to maintain the "vicious circle" of oxidative stress and its harmful metabolic effects. It could be a mediator between the oxidative stress and the inflammation in adipose cells (Zarrouki, Soares, Guichardant, Lagarde & Geloen 2007) and could be involved in the activation of in vivo cellular pathways generating a low rate of inflammation of the organism and alter the function of proteins involved in lipid anabolism and suppression of inflammation of adipose tissue (Russell, Gastaldi, Bobbioni-Harsch, Arboit, Gobelet, Deriaz et al. 2003 ; Zarrouki, Soares et al. 2007). 4-hydroxyalkenals could also intervene in the onset of the metabolic syndrome because of their capacity to alter insulin signalization (Pillon, Soulere, Lagarde & Soulage, 2009; 2010).

Conclusions

Regarding the toxicity of lipid oxidation products, it is sometimes argued that high reactivity does not necessarily imply toxicity or damage under physiological conditions because the compounds are often present in small concentrations in the diet. In addition, the organism has powerful defence mechanisms to neutralize and eliminate the reactive compounds, including oxidoreductases, nucleophilic trapping agents, glutathion dependent enzymes, thereby limiting their potential toxicity. Other protective mechanisms include the high turnover and therefore repair rate of gut epithelial cells, and the presence of powerful hepatic and renal detoxification mechanisms (Baynes, 2007). However, dietary lipid oxidation products must attract much attention because of the wide variety of degenerative processes and diseases associated with oxidative stress. It appears that precise data on toxicity thresholds of lipid oxidation products are not available. Our ongoing research project include *in vitro* studies of the formation of lipid oxidation products, of the absorption of 4-hydroxyalkenals by Caco-2 cells, a model of intestinal epithelium, and their cellular metabolic consequences, and studies in rodents about the effects of oxidized n-3 PUFA oil consumption on oxidative stress and inflammation.

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