

Phenolics against the Oxidation of o/w Emulsions

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Contributor: Sotirios Kiokias, Vassiliki Oreopoulou

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1. Introduction

Lipid oxidation is a serious problem for scientists since it adversely affects the product quality in the food, cosmetic, and pharmaceutical sectors ^[1]. Many common food products exist as oil-in-water (o/w) emulsions including beverages, dressings, sauces, soups, and desserts ^[2]. One of the main causes of the quality deterioration of these products is the oxidation of lipids ^[3]. The degradation of unsaturated lipids can lead to off-flavors, decreases the nutritional profile of foods, and may eventually generate toxic products ^[4]. In emulsified foods, lipid oxidation can occur rapidly due to their large surface area, with mechanisms that are more complex and not fully understood compared to bulk oils ^{[5][6]}. As a consequence, the food industry faces a serious problem due to the low oxidative stability of emulsified systems, which adversely impacts consumer safety and the economic viability of the products ^[7]. A better understanding of the endogenous and exogenous factors that monitor the microstructural and oxidative stability of food emulsions would help to maintain their desirable functional and sensory properties during the formulation, processing, and storage of relevant products ^[8]. In addition, emulsion systems generally mimic the amphiphilic nature of important biological membranes (such as lipoproteins) that are prone to oxidative degradation when attacked by singlet oxygen and free radicals ^[9]. This biochemical process eventually links to the development of serious human health conditions, such as aging, carcinogenesis, and cardiovascular diseases ^[10].

Over the last few years, there is a steady food market trend for the use of natural antioxidants, as a common strategy to slow down lipid oxidation in emulsified foods, increase their shelf life, and minimize bad odors ^{[11][12]}. A body of recent research ^{[13][14]} focuses on nano-emulsions that are increasingly used in various products in order to incorporate easily degradable bioactive compounds, protect them against oxidation, and enhance their bioavailability. In manufactured products (quite often heterogeneous systems containing lipids as emulsions or bulk phase) the efficiency of an antioxidant is determined not only by its chemical reactivity, but also by its physical properties and its interaction with other compounds present in the products ^[15].

With the term phenolics, researchers refer to a wide class of natural compounds (e.g., tocopherols, flavonoids, phenolic acids) with varying structures and antioxidant properties ^[16]. The application of phenolic compounds in various commercial products has attracted an increasing level of interest over the last few years from researchers in the food, pharmaceutical, and nutraceutical industries ^[17]. It is true, though, that certain structural characteristics (including the hydrophobic character of many phenolics) may affect their integration into real products as well as their general biological activities and their bio-absorption in human organisms ^[18]. Their potential to act as functional ingredients upon their addition in bulk or emulsified oils has been an area of emerging scientific interest in the last decade ^{[19][20][21]}. A few researchers in this field have focused on phenolic acids, their activity, health effects, and extraction from natural plant sources ^{[21][22][23]}. Phenolic acids are classified as hydroxybenzoic and hydroxycinnamic acids, and, depending on their structure, may present solubility in water or lipids, thus enabling their use in many products ^{[7][14][24]}.

2. Overview of Studies Reporting on the Effect of Phenolics against the Oxidation of o/w Emulsions

Although most of the studies on phenolic antioxidants so far have focused on bulk oils, a body of recent research has examined the activity of various phenolic compounds in food-related o/w emulsions. In emulsified systems, the initial step of lipid oxidation takes place at the interface between the oil and water phases. Over the last few years, an increasing

number of researchers have reported well-documented antioxidant activities of naturally occurring phenolics in o/w model systems [19][25].

Tocopherols are a class of natural compounds with a high nutritional value in the human diet because of their vitamin E activity. In addition, various authors have reported their well-established antioxidants activity in vitro (oil model systems) and in vivo (human clinical trials) [26][27]. Wang et al. [28], following oxidation experiments of flaxseed o/w emulsions, have noted a higher antioxidant efficiency of δ -tocopherol compared to α -tocopherol. The authors linked the superior activity of δ -tocopherol to its enhanced distribution in the interfacial area of the emulsion system. Another similar study—based on the autoxidation of shrimp o/w emulsions under storage at room temperature did not report any protective effect of α -tocopherol against oxidative destabilisation [29]. On the contrary, α -tocopherol when combined with chitooligosaccharides (at both 0.2 and 0.4 g/L concentrations) significantly delayed the oxidation process, in terms of both primary (conjugated dienes) and secondary (TBARs) oxidation products indicators [29]. Barouh et al. [15] further explored the activity of tocopherols in bulk oils and emulsion systems as a function of various physicochemical parameters (including their interaction with specific emulsifiers such as Tween 65, Tween 80, whey proteins, etc.).

Flavonoids comprise another class of widespread phenolic compounds with well-established antioxidant properties strongly related to their structure [30][31]. Common plant flavonoids such as quercetin, rutin, and hesperitin prolong the shelf life of o/w emulsions by retarding the oxidative deterioration and improving physical stability. However, only quercetin retained its antioxidant activity in the presence of ferric ions due to its structural configuration (3'4' dihydroxy substitution of B ring and the presence of 3-OH) [32]. In fact, quercetin seems to be one of the most potent contributors to the antioxidant activity of plant extracts in emulsions [33]. However, quercetin has been reported to exert a pro-oxidant character in pure lipids and antioxidant activity in emulsions due to its hydroxyl group in position-3 [34]. Catechin acted as a better antioxidant than quercetin in the copper-induced oxidation of diluted, Tween-based, linoleic acid emulsion (0.02 M), while morin presented the best protective action [35]. Different metal ions may affect the activity of flavonoids in emulsions, where the pro-oxidant or antioxidant character may depend on the flavonoid structure and the metal type [36]. Flavonoids have also been examined for the preparation of physically stable pickering emulsions, while also offering oxidative stability [37][38].

Plant extracts are rich in flavonoids and phenolic acids and exert a strong antioxidant activity in o/w emulsions that may be attributed to synergism among its components and also to partitioning in the aqueous phase (e.g., polar phenolic acids or flavonoid glycosides), oil-water interface (e.g., medium polarity phenolic acids and flavonoids), or the oil phase (e.g., flavonoid aglycons) of the emulsion [39][40]. Additionally, the presence of endogenous antioxidants such as α -tocopherol may affect the flavonoid activity in lipid systems; e.g., it showed a strong synergistic effect with quercetin in emulsions, and a clear antagonistic effect in bulk oil [41]. More information on the phenolic synergistic effects is given in original context.

The current research has focused on various phenolic acids available in many natural sources (e.g., in olive oil, herbs, fruits) that have been widely explored in food systems [42]. Caffeic acid (CA) is perhaps the most well-known phenolic acid widely spread in the plant kingdom [43]. CA exerted a clear antioxidant effect when added in o/w emulsion systems stabilised by Tween and prepared with various vegetable oils [44][45]. Ferulic acid (FA) is another phenolic acid -commonly present in many plant seeds [46] that was reported to act as a strong antioxidant following its addition in a range of oil-based emulsions [47][48][49].

For gallic acid (GA), which is found in high amounts in tea and berries [50] there is some contradictory evidence in the literature about its protective action against oxidation of food emulsions. GA and its alkyl esters were found to exert a clear antioxidant effect against the oxidation of rapeseed o/w nano-emulsions stabilised by SDS [51] or even double emulsions by the use of encapsulation [52]. Additionally, Zhu et al. [53] concluded a strong inhibitory effect of GA and its alkyl esters against the formation of both primary (Peroxide Values) and secondary oxidation products (hexanal content). Propyl gallate exerted a superior activity compared to gallic acid and the other gallate esters. Other studies, however, did not report any protective effect of GA in o/w emulsions [54][55].

A large variety of plants and vegetables are abundant in p-coumaric acid (p-CA) [56]. Park et al. [57] reported that roasted rice hull extracts, with a high concentration of p-CA enhanced the oxidative stability of bulk oil and o/w emulsions at 60 °C. Rosmarinic acid (RA) is the main phenolic component in various edible and aromatic herbs of the Lamiaceae family (including *Rosmarinus officinalis*, *Origanum* spp., etc.) [58]. Choulitoudi et al. [19] observed that ethanol and ethyl acetate extracts of *Satureja thymbra*—articularly rich in RA—reduced by 75–80% the oxidation rate of sunflower o/w emulsions at chilling temperature (5 °C). Other researchers reported the antioxidant activity of RA in corn oil- and soyabean oil-based emulsions [59][60] as well as in a model emulsion system based on linoleic acid [44].

Bakota et al. [61] incorporated either pure RA or RA-rich extract (from *Salvia officinalis* leaves), at a concentration of ~30 mg/g, into o/w emulsions and observed that both treatments were effective in suppressing lipid oxidation.

Vanillic acid (VA) is a phenolic acid found in several fruits, olives, and cereal grains. Keller et al. [62] observed a strong antioxidant character of VA during the autoxidation of Tween 40-based o/w systems, at pH 3.5. In addition, a few authors [28][63] reported that the addition of tannic acid (TA) enhanced the resistance of plant-based emulsions against both droplet aggregation and lipid oxidation as a result of its strong ferrous ion-binding properties.

Ascorbic acid is another phenolic compound widely present in nature and with a high nutritional value (also known as vitamin C). A number of scientists highlighted the important role of ascorbic acid in antioxidant synergies examined in both food and clinically relevant studies [64][65].

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