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Formulation and characterization of food-grade microemulsions as carriers of natural phenolic antioxidants

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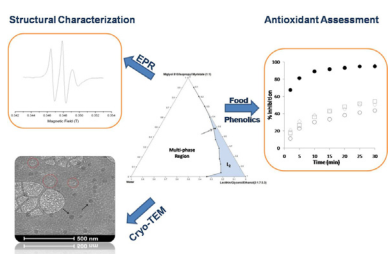
HIGHLIGHTS

- Food-grade W/O microemulsions were developed as carriers of phenolic antioxidants.
- Swollen micelles with diameters smaller than 10 nm were detected by DLS measurements.
- Membrane dynamics were slightly affected by the encapsulation of the antioxidants.
- Cryo-TEM indicated the existence of entangled thread-like reversed micelles.
- Radical scavenging activity of the encapsulated antioxidants was evaluated by EPR.

GRAPHICAL ABSTRACT



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ABSTRACT

Food-grade W/O microemulsions based on lecithin, caprylic/capric triglycerides, isopropyl myristate, alcohols and water were formulated and structurally characterized to be used as potential carriers of natural food antioxidants. Different well-known food antioxidants including gallic acid, p-hydroxybenzoic acid, protocatechuic acid and tyrosol were successfully encapsulated in the aqueous cores of the microemulsions. A pseudo-ternary phase diagram was constructed to determine the extent of the monophasic area that corresponds to an inverted type microemulsion. Apparent hydrodynamic diameter measurements of empty and loaded microemulsions were performed using dynamic light scattering (DLS) and swollen micelles with diameters smaller than 10 nm were detected. Interfacial properties of the microemulsions were studied by electron paramagnetic resonance (EPR) spectroscopy employing the nitroxide spin probe 5-doxylstearic acid (5-DSA). A small increase in spin probe mobility upon addition of the antioxidants was observed; whereas the rigidity of the surfactants was not affected. Cryogenic transmission electron microscopy (Cryo-TEM) indicated the existence of entangled thread-like reversed micelles. Finally, the investigated phenolics were assessed and compared for their radical scavenging activity using an EPR approach based on free radicals. The encapsulated gallic acid showed the highest antioxidant activity (0.93 mM trolox equivalents) as compared to other antioxidants assessed within the frame of this study.

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1. Introduction

Food industry, nowadays, is increasing its interest in the formulation of biocompatible microemulsions to be used as reservoirs of bioactive substances. It is the main aim in this food research area to introduce novel carriers for natural compounds and functional foods that have positive health benefits when consumed in specific concentrations [1]. However, the major challenge is the limited bioavailability or the poor solubility of these bioactive compounds inside the human body. Microemulsions, as food delivery systems, can overcome these problems in a satisfactory level due to their unique structural characteristics [2].

Microemulsions are isotropic thermodynamically stable solutions characterized by their self-assembled structure, their spontaneous formation and low viscosity [3]. The droplet size of the swollen micelles ranges from 5 to 50 nm. They are formed due to the self-assembly process of the surfactant molecules, consisting of hydrophilic headgroups and hydrophobic tails. Microemulsions contain at least three main components: an oil, an emulsifier, and water [4]. There are many advantages of microemulsions over conventional dispersions such as emulsions and double emulsions as carriers of bioactive molecules owing to their unique properties. For instance, microemulsions are easy to prepare and scale up for commercial applications; they have a long shelf life; and also offer the possibility of improved solubilization and protection of encapsulated compounds. Microemulsions have found numerous applications over a wide range of areas such as pharmaceuticals, foods, cosmetics, agrochemicals, digestion model and new applications are continuously being reported [5–8].

Microemulsions used in food industry face limitations mainly due to the restrictions in surfactants' consumption and a possible toxicity of hydrophobic constituents [9]. Many surfactants are entirely prohibited for food applications by either the Food and Drug Administration or the European Food and Safety Authority (EFSA), while others are permitted in very low concentrations. Food-grade emulsifiers such as lecithin, monoglycerides and diglycerides, among the surfactants, medium chain triglycerides (MCT), isopropyl myristate (IPM) [10] among the oils, ethanol (at low concentrations) and glycerol are some of the permitted materials of the EFSA and are generally recognized as safe (GRAS).

Caprylic/capric triglycerides (Miglyol 810) belong to a class of vegetable oils in which three medium chain saturated fats are bound to the hydrophilic glycerol backbone. MCTs are distinguished from other triglycerides in that each fat molecule consists of medium chain length containing between six to twelve carbon atoms. Isopropyl myristate is an oil composed of isopropyl alcohol and myristic acid (a naturally-occurring fatty acid) widely used as a cosmetic and pharmaceutical ingredient. Soybean lecithin is a combination of naturally-occurring phospholipids, extracted during the processing of soybean oil. It has been successfully used for the construction of various biocompatible microemulsion formulations [11].

Bioactive compounds with scavenging activity against stable free radicals are extremely beneficial in every day diet. Food-grade microemulsions filled with antioxidants have been proved to prevent quality deterioration of products and to maintain their nutritional value [12]. Antioxidants have also been of interest to biochemists and health professionals because of their important role in protecting the body from severe damage caused by reactive oxygen species [13].

In general, phenolic acids, including gallic acid, 4-hydrobenzoic acid, and protocatechuic acid (Fig. 1) are found in a variety of plant derived foods [14]. Phenolic acids have been associated with sensory qualities and also nutritional and antioxidant properties of foods [15]. As it has been well established in the literature, health effects of polyphenols depend on the consumed amount and also on their bioavailability [16]. Gallic acid, in particular, is a natural antioxidant, which has antiallergic, antimutagenic, anti-inflammatory, and anti-carcinogenic activities [17–20]. It belongs to the class of phenolics that are food additives being claimed as beneficial for human health [21].

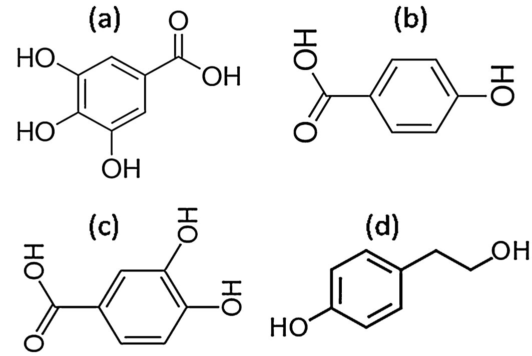

Fig. 1. Chemical structures of (a) gallic acid, (b) p-hydroxybenzoic acid, (c) protocatechuic acid and (d) 2-(4-hydroxyphenyl) ethanol.

In addition, tyrosol (2-(4-hydroxyphenyl) ethanol) (Fig. 1) is a natural phenolic antioxidant present in a variety of natural sources. The principal source in the human diet is olive oil. Although it is not as potent as other antioxidants present in olive oil, its higher concentration and good bioavailability indicate that it may have an important overall effect [22].

The aim of the present study was to report on the formulation of a food-grade microemulsion based on lecithin, MCT, IPM, ethanol and glycerol as carriers of different antioxidants with potential positive health effects. The development of such systems could be potentially considered by food industries in the design and development of novel functional food formulations. Since modification or fortification of different food products focusing on different areas of health concern are of growing interest among the various sectors of food industry, the proposed research results could be of particular interest.

Structural characterization of the proposed system was obtained using dynamic light scattering (DLS), electron paramagnetic resonance (EPR) spectroscopy *via* spin probing technique, and cryogenic-transmission electron microscopy (Cryo-TEM) [23–25]. Moreover, a comparative assessment for the antioxidant activity based on EPR spectroscopy of the stable galvinoxyl free radical was considered in order to evaluate the radical scavenging activity of the antioxidants when encapsulated in the compartmentalized environment of the microemulsions [26].

2. Materials and methods

2.1. Materials

The oil MIGLYOL®810, which is medium chain triglyceride (MCT), was purchased from CRÈMER Oleo Division, Germany. Soybean lecithin (Emulmetic 930) containing 92% phosphatidylocholine was supplied from Lucas Mayer, Germany. Isopropyl Myristate (IPM) (95%) was provided from Fluka, Switzerland. Gallic acid (99.3%), p-hydroxybenzoic (99%), protocatechuic acid (97%) were obtained from Sigma–Aldrich, Germany. 2-(4-Hydroxyphenyl ethanol) was from Fluka, Switzerland. 6-Hydroxy-2,5,7,8 tetramethylchromano-2-carboxylic acid (Trolox) and the stable free radical Galvinoxyl were purchased from Sigma–Aldrich, Germany. Isooctane was from Merck, Darmstadt, Germany. Glycerol was from SERVA, Germany. Ethanol (≥98%) was obtained from Sigma Aldrich, Germany. Highly purified water was obtained from a Millipore Milli Q Plus device.