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Lipid oxidation in base algae oil and water-in-algae oil emulsion: Impact of natural antioxidants and emulsifiers

Bingcan Chen [a,*], Jiajia Rao [a], Yangping Ding [d], David Julian McClements [b,c], Eric Andrew Decker [b,c,**]

[a] Department of Plant Sciences, North Dakota State University, Fargo, ND 58108, USA
[b] Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA
[c] Bioactive Natural Products Research Group, Department of Biochemistry, Faculty of Science, King Abdulaziz University, P. O. Box 80203, Jeddah 21589, Saudi Arabia
[d] College of Food Science, Southwest University, Beibei, Chongqing 400715, China

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ABSTRACT

The impact of natural hydrophilic antioxidants, metal chelators, and hydrophilic antioxidant/metal chelator mixture on the oxidative stability of base algae oil and water-in-algae oil emulsion was investigated. The results showed that green tea extract and ascorbic acid had greatest protective effect against algae oil oxidation and generated four day lag phase, whereas rosmarinic acid, grape seed extract, grape seed extract polymer, deferoxamine (DFO), and ethylenediaminetetraacetic acid (EDTA) had no significant protective effect. Besides, there was no synergistic effect observed between natural antioxidants and ascorbic acid. The emulsifiers are critical to the physicochemical stability of water-in-algae oil emulsions. Polyglycerol polyricinoleate (PGPR) promoted the oxidation of emulsion. Conversely, the protective effect on algae oil oxidation was appreciated when defatted soybean lecithin (PC 75) or defatted lyso-lecithin (Lyso-PC) was added. The role of hydrophilic antioxidants in emulsion was similar to that in algae oil except EDTA which demonstrated strong antioxidative effect in emulsion. The results could provide information to build up stable food products containing polyunsaturated fatty acids (PUFA).

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1. Introduction

Numerous studies have found an association between the dietary long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) and the health benefits (Dangardt et al., 2010; Saito et al., 2008). The potential benefit of n-3 PUFA consumption is to prevent, ameliorate, or even treat cardiovascular disease (CVD) (Calder, 2004; Herold & Kinsella, 1986). Given these obvious merits of n-3 PUFA, there is a trend to enrich foods with n-3 PUFA. Ideally, the benefits of PUFA for human health can be accomplished by incorporating beneficial PUFA into food products, such as infant formula, butter, milk, mayonnaise, ice cream, energy bar, and yoghurt (Chen, McClements, & Decker, 2012). However, chemical degradation is a major issue for incorporating PUFA into food products because they are more susceptible to oxidation than the oils having less double bond (Decker, Akoh, & Wilkes, 2012). The instability of PUFA is often accelerated by the factors such as the abuse of heat, the over exposure to the light, and the contamination of transition metals. Such factors can barely avoid during food processing and storage and thus lead to complex autoxidation, photooxidation, or thermal oxidation. The oxidation of PUFA could not only result in the loss of their potential benefits but also cause the development of off-flavors, the loss of other nutrients and the formation of potentially toxic compounds which could threaten human health. Thus, preventing the oxidation of labile PUFA in its bulk state or in real foods is the foremost obstacle to maintain their benefits. In this matter, different strategies have been proposed and developed to delay the oxidation in foods imparted by the addition of PUFA, and the employment of antioxidants is the most efficient means to retard lipid oxidation (Shahidi & Zhong, 2010).

Although synthetic antioxidants may generate greater antioxidant efficacy, the use of such has been strictly regulated due to their potential carcinogenic and toxic effects as indicated by cellular or animal studies (Botterweck, Verhagen, Goldbohm, Kleinjans, & Van Den Brandt, 2000; Branen, 1975). Consequently, the addition of natural antioxidants is the prevailing mean to prevent PUFA oxidation before being consumed. However, one current challenge complicating the successful application of such is the limitations and the relatively low activity of approved natural antioxidants for foods with labile PUFA. It is also difficult to predict the antioxidant activity since many factors could impact antioxidant efficacy, such as their polarity, the type of food matrices they embed, and the coexistence of other antioxidant(s). For instance, earlier study suggested that the hydrophilic antioxidants work better in bulk oil than in emulsion systems (Frankel, Huang, Kanner, &

* Corresponding author.
** Correspondence to: E.A. Decker, Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA.
E-mail addresses: bingcan.chen@ndsu.edu (B. Chen), edecker@foodsci.umass.edu (E.A. Decker).

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German, 1994). Lately studies show that the antioxidative effectiveness of α-tocopherol and its hydrophilic analog Trolox cannot only affect by their concentrations but highly rely on the naturally existed association colloids in soybean oil which is inextricably intertwined with ~300 ppm water (Chen, Han, Laguerre, McClements, & Decker, 2011). In addition, moisture in oils plays an important role as a substrate for the lipid oxidation and high moisture content accelerates the decomposition of α-tocopherol (Kim, Kim, Yi, Oh, & Lee, 2015; Kim, Yi, Kim, & Lee, 2014).

Algae oil is a major source of n-3 PUFA and the main omega-3 fatty acid, DHA in base algae oil can reach to 50%. Unlike bulk vegetable oil, the water content in algae oil is extremely low (less than 50 ppm). The antioxidants applied in such oil include mixed tocopherol, ascorbyl palmitate, lecithin, and rosemary extract, all of which are hydrophobic antioxidants. The fate of hydrophilic antioxidants in such oil, however, is unpredictable so far. Therefore, the purpose of this research is to study the effectiveness of several popular hydrophilic antioxidants in the algae oil and water-in-algae oil emulsions. In addition, the role of emulsifiers on the chemical stability of water-in-algae oil emulsion will also be examined.

2. Materials and methods

Base algae oil without endogenous antioxidants and green tea extract (>90% epigallocatechin gallate, Teavigo®) were obtained from DSM Nutritional Products Ltd (Columbia, MD). Grape seed extract and grape seed extract polymer (>90% polyphenols, MegaNatural®-BP) were obtained from Polyphenolics Inc. (Madera, CA). Rosmarinic acid, α-tocopherol, deferoxamine (DFO), and ascorbic acid were purchased from Sigma-Aldrich Co. Ethylenediaminetetraacetic acid (EDTA) disodium salt was purchased from Chempure Ultra (Houston, TX). Polyglycerol polyricinoleate (PGPR, 4110, HLB ≈ 4.3) was obtained from Palsgaard (Morristown, NJ). Mono-Diglycerides (Grindsted® Mono-Di Hv-52KA, MAG-DAG, HLB ≈ 5–6) was a gift from Danisco (Danisco USA Inc.). Defatted soybean lecithin PC75 and PC50 containing 75% and 50% of phosphatidylcholine respectively, were obtained from American Lecithin Co. (Oxford, CT). Defatted lyso-lecithin (Solec 8160) was obtained from the Solae Company (St Louis, MO). HPLC grade methanol and n-hexane were obtained from Fisher Scientific (Pittsburgh, PA).

2.1. Preparation of base algae oil with hydrophilic antioxidants

Total phenolic contents in hydrophilic antioxidants were determined using a spectrophotometric technique based on the Folin-Ciocalteau reagent (Georgé, Brat, Alter, & Amiot, 2005), and the values hereafter were expressed using units of μM on the basis of gallic acid equivalents (GAE). The hydrophilic antioxidants and/or metal chelators (50 μM) were firstly dissolved into methanol. Then, the required volume of antioxidant solution was pipetted into a beaker to reach the desired concentration (i.e., 100, 300, and 500 μM) after which was flushed by dry nitrogen. Algae oil was weighted using the beakers containing antioxidants, followed by the sonication in ice water ultrasonic bath (Branson Ultrasonic Corporation, Danbury, CT) for 10 min to fully dissolve the antioxidants. The algae oil was finally stirred at 500 rpm for 8 h at a 4 °C incubator room.

One milliliter of fresh made algae oil was transferred to GC vials using an Eppendorf® adjustable pipet along with a 12.5 mL Plastibrand PD-TIP (Wertheim, Germany) to ensure the accuracy and precision. The vials were then sealed by screw thread caps with inserted polytetrafluoroethylene (PTFE)/butyl rubber septa. For lipid oxidation studies, all vials were stored in a close box and stayed in a 45 °C incubator room in the dark for up to 10 days and short storage time may be reported when the oxidative kinetic went to exponential phase.

2.2. Preparation of water-in-algae oil (w/o) emulsion with hydrophilic antioxidants

The antioxidants and/or metal chelators were dissolved directly into the double distilled water before mixing with algae oil. This solution was referred to as the stock antioxidants solution.

Surfactant solution (30 wt%) was prepared by heating the mixture of 6 g surfactant (PGPR, PC75, PC50, lyso-PC and MAG-DAG) and 14 g medium chain triacylglycerides (MCT) at 65 °C to ensure complete dispersion. Stock algae oil was prepared by dispersing surfactant solution (0.5 wt%) into algae oil. A 2 wt% water-in-algae oil emulsion was then prepared by homogenizing antioxidants solution (2 wt%) and stock algae oil (98 wt%) using a high-speed blender (M133/128-0, Biospec Products, Inc., ESGC, Switzerland) for 2 min followed by further homogenization with a Microfluidizer (model M-110 L Microfluidizer Processor, Microfluidics, Newton, MA) for three passes at a pressure of 68 MPa. The sample without antioxidants solution (0 wt% water) prepared by the same procedure was used as a blank. The samples were kept in an ice container during the whole procedure to minimize oxidation.

Sample vial preparation for lipid oxidation studies followed the previous procedure, and stored in a 45 °C incubator room in the dark for up to 10 days. For storage stability study, 10 mL of sample were transferred into a test tube (internal diameter 15 mm, height 125 mm), tightly sealed with a plastic cap, and then stored at room temperature. Photographs of the emulsions were recorded after 24 h using a digital camera. The microstructure of selected emulsions was evaluated using optical microscopy after 24 h (Nikon microscope Eclipse E400, Nikon Corporation, Japan).

2.3. Measurement of lipid oxidation products

Lipid hydroperoxides were measured as the primary oxidation product using a method adapted from Shanta and Decker (Shantha & Decker, 1994). Secondary oxidation product marker propanal was monitored using a GC-17A Shimadzu gas chromatograph bundled with an AOC-5000 autosampler (Shimadzu, Kyoto, Japan). Oil samples (1 mL) in 10 mL capped GC glass vials were preheated at 45 °C for 15 min in AOC-5000 autosampler heating block. A 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane solid-phase microextraction (SPME) fiber needle (Supelco, Bellefonte, PA) was injected into the vial absorbing volatiles for 2 min, and then was transferred to the injector port (250 °C) for 3 min. Split mode was selected at the ratio of at 1:5 for the injection port. Volatiles were identified on a Supelco 30 m × 0.32 mm Equity DB-1 column with a 1 μm film thickness at constant 65 °C for 10 min. The carrier gas was helium and the flow rate was 15.0 mL/min. A flame ionization detector (FID) was set at a temperature of 250 °C. The concentration of propanal was calculated from its peak areas using a standard curve prepared from an authentic standard. The lag phase is defined as the time required to observe a sudden increase of propanal formation.

2.4. Determination of tocopherol concentration in base algae oil

The concentration of tocopherol in base algae oil during storage time was determined by normal phase HPLC. For HPLC analysis, weighted oil sample (approximately 0.1 g) was dissolved in 5 mL hexane. The solution was passed through a 0.45 μm filter after vortexing. A 20 μL aliquot of the solution was separated using a Shimadzu (10A, Shimadzu Co., Kyoto, Japan) HPLC system with a 250 mm × 4.6 mm i.d., 5 μm, ZORBAX Rx-SIL analytical column. The mobile phase of the HPLC system consisted of hexane:isopropyl alcohol (99:1 v/v) using isocratic gradient at a flow rate of 1 mL/min. Column temperature was maintained at 40 °C. Detection of tocopherol was conducted using both a Shimadzu diode array detector (DAD) at 295 nm, and a Waters 474 scanning fluorescence detector at an excitation wavelength of 290 nm and an

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