Food Chemistry 178 (2015) 1–9

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Oxidative stability of structured lipid-based infant formula emulsion: Effect of antioxidants

Long Zou, Casimir C. Akoh *

Department of Food Science and Technology, The University of Georgia, Athens, GA 30602, USA

ARTICLE INFO

Article history:
Received 11 August 2014
Received in revised form 8 December 2014
Accepted 13 January 2015
Available online 21 January 2015

Keywords:
Lipid oxidation
Antioxidant
Structured lipid
Infant formula
Emulsion

ABSTRACT

The effect of permitted antioxidants, including α-tocopherol, β-carotene, ascorbyl palmitate, ascorbic acid, citric acid, and their combinations, on the lipid oxidation of structured lipid (SL)-based infant formula (IF) was evaluated. The 3.5% oil-in-water IF emulsion was formulated with a human milk fat analogue enriched with docosahexaenoic acid and stearidonic acid, and the antioxidants were added at 0.005% and 0.02% of the oil. The peroxide value, anisidine value, and hexanal concentration of emulsion samples were measured over a 28-day period. The results showed that whether a compound exhibited antioxidant behavior depended on its mechanism of action, polarity, concentration, and environmental conditions. The most effective antioxidant was ascorbyl palmitate at 0.005%, and a synergistic antioxidant effect was found between α-tocopherol and β-carotene. A high correlation was observed between anisidine value and hexanal content. Our findings have important implications for the successful incorporation of SL into IF products for infant nutrition and health.

1. Introduction

With the increased use of polyunsaturated oils and fortification of mineral nutrients (e.g., iron) for health benefits, lipid oxidation has become a major concern in emulsion-based food products, such as milk, beverages, and ready-to-feed infant formula (IF). It leads to the development of undesirable off-flavors, nutrient loss, and potentially toxic compounds, thus making the foods unsuitable for consumption (McClements & Decker, 2000). One of the most effective and convenient strategies to retard or prevent lipid oxidation is to add antioxidants (Shahidi & Zhong, 2010).

According to the mechanisms of action, antioxidants can be broadly classified as primary antioxidants which scavenge free radicals to break chain-reactions of oxidation, or secondary antioxidants which protect lipids against oxidation mainly by chelating transition metals, quenching singlet oxygen, replenishing hydrogen to primary antioxidants, and/or scavenging oxygen (Reische, Lillard, & Eitenmiller, 2008). According to the Codex Alimentarius regulations (CAC, 1981), tocopherols, β-carotene, ascorbic acid, ascorbyl palmitate, and citric acid are permitted compounds that may act as antioxidants in regular milk-based IF. α-Tocopherol is an important lipophilic primary antioxidant that donates phenolic hydrogen to lipid free radicals (Eitenmiller & Lee, 2004). Due to the presence of conjugated double bonds in the molecule, the lipid-soluble β-carotene can act as primary antioxidant by scavenging free radicals under low oxygen pressure or as secondary antioxidant by physically quenching singlet oxygen to produce ground-state triplet oxygen and release the excess energy in the form of heat (Reische et al., 2008). Water-soluble ascorbic acid represents a multifunctional antioxidant that exerts its antioxidant effect via inactivation of free radicals, regeneration of primary antioxidant by hydrogen donation, metal chelating, and scavenging oxygen by reduction (Reische et al., 2008). Ascorbyl palmitate is the hydrophobic derivative of ascorbic acid, but its antioxidant ability is still based on the ascorbic acid moiety. Citric acid with multiple carboxyl groups is capable of inhibiting metal-catalyzed oxidation by forming a thermodynamically stable complex with transition metal ions (Decker, 2008). Moreover, combinations of antioxidants with different mechanisms of action, such as α-tocopherol/ascorbic acid, α-tocopherol/citric acid, and α-tocopherol/β-carotene, have been reported to exhibit synergistic antioxidant effects (Decker, 2008; Eitenmiller & Lee, 2004). Kamal-Eldin and Appelqvist (1996) proposed two major mechanisms by which synergism to tocopherols can be explained. First, a tocopherol sparing effect occurs when another antioxidant working by the same or a different mechanism is present, which can be a free radical scavenger or singlet oxygen quencher (e.g., β-carotene) or metal chelator (e.g., citric acid). Second, tocopherols are regenerated from tocopheroxyl radicals by another antioxidant with hydrogen donation ability (e.g., ascorbic acid).

* Corresponding author. Tel.: +1 706 542 1067; fax: +1 706 542 1050.
E-mail address: cakoh@uga.edu (C.C. Akoh).

http://dx.doi.org/10.1016/j.foodchem.2015.01.073
0308-8146/

Oil-in-water (O/W) emulsion is often more susceptible to oxidation than bulk oil due to its larger surface area that promotes interactions between the lipids and water-soluble prooxidants (Waraho, McClements, & Decker, 2011). Oxidative reactions are believed to be mostly prevalent at the oil–water interface (McClements & Decker, 2000). Therefore, in addition to its innate potency, the effectiveness of an antioxidant is influenced by its polarity and solubility, which subsequently determine its actual location in the O/W emulsion. With respect to interfacial phenomenon, "polar paradox theory" was proposed, which states that polar antioxidants are more effective in less polar media, such as bulk oil, whereas nonpolar antioxidants are more effective in more polar media, such as O/W emulsions (Porter, 1993). The high efficacy of nonpolar antioxidants in O/W emulsions is primarily attributed to their high affinity to orient toward the oil–water interface. However, new evidence suggests that this empirical observation seems to be a particular case of a much wider picture, and more complex factors (e.g., critical concentration) in addition to polarity should be considered to explain antioxidant efficacy (Shahidi & Zhong, 2011). Furthermore, compounds with antioxidant activity may also exhibit prooxidant behavior under certain conditions (Osborn-Barnes & Akoh, 2003b). Huang, Frankel, and German (1994) reported that whether α-tocopherol acted as an antioxidant or prooxidant depended on the test system, concentration, oxidation time, and the method used to determine oxidation. Therefore, all relevant factors must be taken into account when selecting antioxidants for a particular food system, and experiments should be performed before adding them as functional ingredients.

In our previous study (Zou & Akoh, 2013b), a human milk fat analogue enriched with docosahexaenoic acid (DHA) and stearidonic acid (SDA), which has potential to benefit the development and growth of infants, was synthesized and incorporated into an actual food system-ready-to-feed IF containing dairy proteins, lactose, lipid, vitamins, minerals, and other nutrients. The emulsifiers and thickeners were optimized to achieve the highest physical and oxidative stability. However, since lipid oxidation in O/W emulsions cannot be controlled by just the emulsifiers and thickeners, antioxidants are needed to further protect against rancidity, and their effectiveness in IF with complex matrices (e.g., metals and emulsifiers), which may interact with antioxidants and affect the rate of oxidation, is necessary to be assessed. Moreover, the oxidative stability of structured lipids (SLs) in real foods has seldom been investigated in the literature (Martin, Reglero, & Senorans, 2010). Therefore, as a continuation of our previous work, the objective of this study was to evaluate the effect of permitted antioxidants, including α-tocopherol, β-carotene, ascorbic acid, ascorbyl palmitate, and citric acid, on the lipid oxidation of SL-based IF emulsions (O/W). Mixtures of α-tocopherol/ascorbic acid, α-tocopherol/ascorbyl palmitate, α-tocopherol/citric acid, and α-tocopherol/β-carotene, were also tested to clarify the mechanism of antioxidant synergism.

2. Materials and methods

2.1. Materials

Tripalmitin was purchased from TCI America (Portland, OR, USA). SDA soybean oil was kindly donated by Monsanto Co. (St. Louis, MO, USA). DHA single-cell oil containing 40% DHA was purchased from DSM Nutritional Products Ltd. (Columbia, MD, USA). *sn*-1,3 Specific lipase Lipozyme TL IM and nonspecific lipase Novozym 435 were obtained from Novozymes A/S (Bagsvaerd, Denmark). α-Tocopherol (⩾96% purity), β-carotene (95% purity), ascorbic acid, ascorbyl palmitate, citric acid (⩾99.5% purity), C7–C30 saturated alkanes, and Tenax porous polymer adsorbent (60–80 mesh) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Hexanal (98% purity) and n-butyl acetate (⩾99.5% purity) were obtained from Alfa Aesar (Ward Hill, MA, USA). Commercial milk-based Similac Advance ready-to-feed IF (Abbott Nutrition, Lake Forest, IL, USA) was purchased from a local convenience store. The DHA and SDA containing SL, which mimicked human milk fat, was enzymatically produced as previously reported (Zou & Akoh, 2013a). All the solvents were of analytical grade, and purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA) or Fisher Scientific (Norcross, GA, USA).

2.2. Preparation of emulsion samples

The SL-based IF emulsion consisting of 3.5% lipid at pH 6.8 was prepared according to the optimal formulation and procedures described by Zou and Akoh (2013b). Antioxidants were added to the emulsion system at 0.02% or 0.005% of the oil. Mixed antioxidant systems contained equal amounts of each compound (0.01% of the oil). α-Tocopherol in hexane was added directly to the oil phase before homogenization. Ascorbyl palmitate was dissolved in ethanol and then added to the oil. β-Carotene was dissolved in hexane before addition to the oil. Organic solvents were evaporated under nitrogen. Ascorbic acid and citric acid were added to the aqueous phase before homogenization. The sample without addition of antioxidants was used as a "control," whereas the sample without addition of antioxidants but flushed under nitrogen was used as another control, named "control-N_2." The commercial ready-to-feed IF was regarded as a "reference."

2.3. Oxidation experiments

The oxidative stability was studied by means of an accelerated storage, which is commonly used to obtain reliable information on product stability. Emulsion samples (50 ml) were placed in capped glass bottles, covered with Parafilm, and allowed to oxidize in a covered water bath at 37 °C for 4 weeks. The primary and secondary oxidation products were measured after 0, 7, 14, 21, and 28 days of storage.

2.4. Spectroscopic measurements of lipid oxidation products

Lipid hydroperoxide, measured as peroxide value (PV), was determined using a modified method of Shantha & Decker, 1994. The analytical procedure was performed as described previously (Zou & Akoh, 2013b). *p*-Anisidine value (AV) determines the amount of aldehydes (mainly 2-alkenals and 2,4-alkadienals), which are secondary products from the decomposition of hydroperoxides. The AV of emulsion samples (2 ml) was determined according to the AOCS Official Method Cd 18–90 (AOCS, 2009) with minor modifications as reported previously (Zou & Akoh, 2013b).

2.5. Identification of volatile compounds by dynamic headspace gas chromatography–mass spectrometry (GC–MS)

Volatile compounds after storage were analyzed using a modified method described previously (Yang, Lee, Jeong, Kim, & Kays, 2008). Briefly, emulsion samples (100 ml) were stirred in a specially constructed 1 l glass beaker at 25 °C for 30 min. The beaker was sealed with a ground glass lid containing entry and exit ports, which were wrapped with aluminum foil to minimize losses during sampling. Immediately after stirring, volatiles were then collected on a 10 cm × 4 mm i.d., stainless-steel Tenax trap (Scientific Instrument Services, Inc., Ringoes, NJ, USA) filled with 250 mg Tenax porous polymer adsorbent, using nitrogen as a purging gas at 100 ml/min for 60 min. A 50 ml Erlenmeyer flask was