Food Research International 99 (2017) 936–943

Contents lists available at ScienceDirect

Food Research International

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

Bioaccessibility and intestinal cell uptake of astaxanthin from salmon and commercial supplements

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ARTICLE INFO

Article history:
Received 17 August 2016
Received in revised form 10 October 2016
Accepted 10 October 2016
Available online 12 October 2016

Keywords:
Astaxanthin
Wild salmon
Aquacultured salmon
Krill oil
Canthaxanthin
Bioaccessibility
Caco-2 cells

ABSTRACT

Although the keto-carotenoid astaxanthin (Ast) is not typically present in human plasma due to its relative scarcity in the typical diet, global consumption of salmon, the primary source of Ast in food, and Ast supplements continues to increase. The first objective of the present study was to investigate the bioaccessibility of Ast from uncooked and cooked fillets of wild and aquacultured salmon, Ast-supplements and krill oil, during simulated gastric and small intestinal digestion. Uptake of *E*-Ast from micelles generated during digestion of wild salmon by monolayers of Caco-2 was also monitored. Both wild and aquacultured salmon flesh contained *E*-Ast and *Z*-isomers of unesterified Ast, whereas Ast esters were the predominant form of the carotenoid in commercial supplements and krill oil. Flesh from wild salmon contained approximately 10 times more Ast than aquacultured salmon. Common styles of cooking flesh from wild and aquacultured salmon decreased Ast content by 48–57% and 35–47%, respectively. Ast in salmon flesh, supplements and krill oil was relatively stable (>80% recovery) during in vitro digestion. The efficiency of transfer of Ast into mixed micelles during digestion of uncooked wild salmon was 43%, but only 12% for uncooked acquacultured salmon. Cooking wild salmon significantly decreased Ast bioaccessibility. The relative bioaccessibility of Ast (41–67%) after digestion of oil vehicle in commercial supplements was inversely proportional to carotenoid content (3–10 mg/capsule), whereas bioaccessibility of endogenous Ast in phospholipid-rich krill oil supplement was 68%. >95% of Ast in mixed micelles generated during digestion of supplements and krill oil was unesterified. Caco-2 intestinal cells accumulated 11–14% of *E*-Ast delivered in mixed micelles generated from digested wild salmon. Apical uptake and basolateral secretion of *E*-Ast by Caco-2 cells grown on inserts were greater after digestion of Ast-enriched krill oil compared to uncooked wild salmon. These data suggest that the bioaccessibility of Ast in wild salmon and soft-gel capsules is greater than that in aquacultured salmon, and that uptake and basolateral secretion of the carotenoid by enterocyte-like cells is enhanced by the digestion products of phospholipid-rich krill oil.

1. Introduction

Salmon is the second most-popular fish consumed in North America. Both wild salmon (*Oncorhynchus* species) and aquacultured salmon (*Salmo salar*) are commercially available. Sales of aquacultured salmon are more than twice that of wild salmon and continue to increase with the expansion of international markets (Knapp, 2013; Knapp, Roheim, & Anderson, 2007). Flesh from wild salmon is naturally pink to red due to the accumulation of the keto-carotenoid astaxanthin (Ast; 3,3′-dihydroxy-β, β-carotene-4,4′-dione; Fig. 1A) resulting from consumption of Ast–rich microalgae and crustaceans. In contrast, flesh from aquacultured salmon is pigmented by inclusion of industrially synthesized Ast and canthaxanthin (Cxn) in feed. Industrial synthesis of Ast yields a product that contains reaction intermediates and optical and stereo-isomers that are not present in natural sources. The development of food plants that have been metabolically engineered to synthesize and accumulate Ast may provide alternative natural sources of the carotenoid. These plant foods include potatoes (Gerjets & Sandmann, 2006), carrots (Jayaraj, Devlin, & Punja, 2008), tomato (Huang, Zhong, Liu, Sandmann, & Chen, 2013) and lettuce (Harada et al., 2014).

In addition to its potent anti-oxidant activity, Ast has been reported to have immunomodulatory, anti-inflammatory, anti-cancer, anti-diabetic, anti-hypertensive, anti-aging and cardio- and ocular-protective effects (Ambati, Phang, Ravi, & Aswathanarayana, 2014; Fassett & Coombes, 2012; Yuan, Peng, Yin, & Wang, 2011). Such activities suggest that Ast and/or its metabolites are delivered to target tissues and are potentially useful for the treatment of several pathological disorders. Like all carotenoids, Ast is hydrophobic and its delivery to tissues requires release from the food matrix and incorporation into mixed micelles during digestion in the small intestine. These particles provide the carotenoid with access to the apical surface of absorptive epithelial cells. The carotenoid is then incorporated into chylomicrons and secreted into the lymph for distribution to peripheral tissues where it may contribute to health promoting activities (Harrison, 2012).

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http://dx.doi.org/10.1016/j.foodres.2016.10.010
0963-9969/

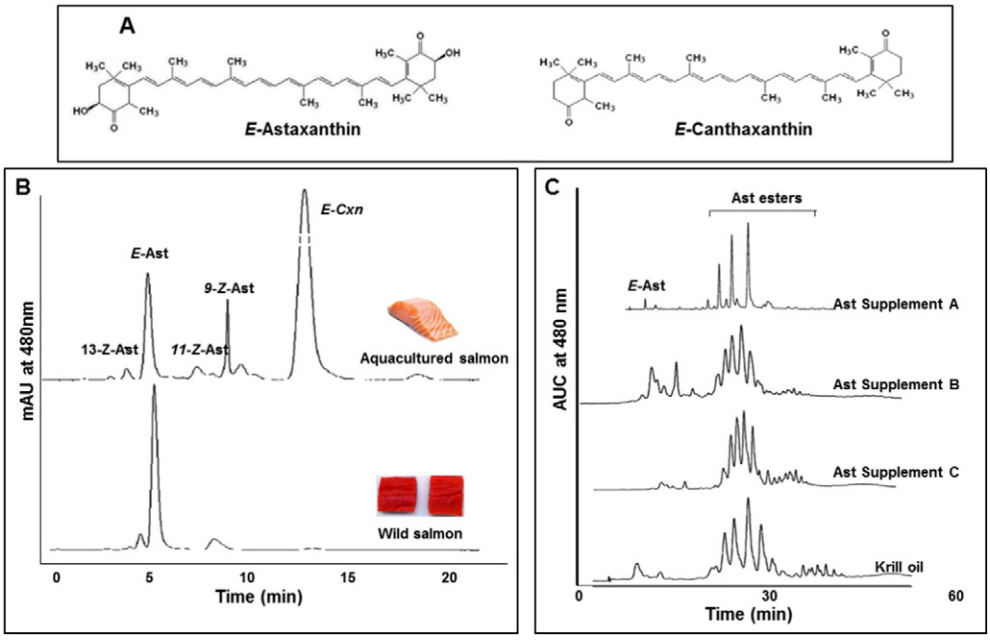

Fig. 1. Panel A) Chemical structures of all *E*-astaxanthin and all *E*-canthaxanthin. Panel B) Representative chromatograms of carotenoids extracted from wild and aquacultured salmon flesh at 480 nm. Upper trace shows separation and detection of carotenoids in extract from uncooked acquacultured salmon. Lower trace is the chromatogram for identically extracted carotenoids from fillets of uncooked wild salmon. Panel C: Representative chromatograms of carotenoids extracted from Ast supplements A, B and C and krill oil. According to the commercial label, the source of Ast for supplements A, B and C was an extract from the microalga *Haematococcus pluvalis*. Other listed ingredients included safflower oil, gelatin, glycerin, and vitamin E. Capsules containing krill oil also contained glycerin, vitamin E, ethyl vanillin, sorbitol and water.

The primary objective of the present study was to investigate the bioaccessibility of Ast in uncooked and cooked fillets from wild and aquacultured salmon, several Ast supplements, and a krill oil supplement. This was accomplished using a well-established, static model of gastric and small intestinal digestion (Chitchumroonchokchai, Schwartz, & Failla, 2004). Apical uptake of all *E*-Ast in micelles generated during the simulated digestion of uncooked and cooked wild salmon by Caco-2 human intestinal cells was also determined. Finally, Caco-2 cells were exposed to mixed micelles generated during the digestion of uncooked wild salmon and Ast-enriched krill oil to compare the effect of the dietary source on the bioaccessibility and the uptake and basolateral secretion of *E*-Ast.

2. Materials and methods

2.1. Materials

Fresh fillets of wild Sockeye salmon (*Oncorhynchus nerka*) was purchased from World Food Market in Columbus, Ohio. Aquacultured Atlantic salmon (*Salmo salar*) was purchased at the World Food Market and two additional area markets and Ast content was initially screened. Fillets from the commercial source with the highest concentration of Ast were selected for comparative studies with wild salmon. Unless stated otherwise, all chemical reagents and general supplies were purchased from Sigma Chemical and Fisher Scientific companies. Acetone-precipitated porcine pancreatin powder (Sigma P4251; Lot O16H7115) was used during small intestinal digestion we have found that carboxyl ester lipase activity (also known as cholesterol esterase) in this preparation greatly exceeds that in the standard pancreatin preparation (Sigma P1750) (unpublished data). Dulbecco's minimal essential medium (DMEM) with 4.5 g glucose/L, L-glutamine (200 mM), non-essential amino acids (NEAA), amphotericin B, gentamicin and fetal bovine serum were purchased from Invitrogen. Three different commercial soft gel capsules containing Ast (products A, B and C), krill oil in soft gel capsules and *Haematococcus pluvialis* extract were purchased from a local nutritional health store in Columbus, Ohio. Ast content of an aliquot of krill oil was increased to 2.5 mg Ast equivalents/mL by addition of Ast extracted and purified (95% purity) from *H. pluvialis* (Praveenkumar et al., 2015) The final product contained 4% unesterified Ast and 96% Ast esters. Following addition of the Ast preparation to krill oil, tubes containing the mixture were placed on rotating mixer for 18 h at 4 °C prior to sonication for 45 s. The mixture was centrifuged (12,000 × *g* at 4 °C, 10 min) and the clear red supernatant was blanketed with nitrogen gas and stored at −80 °C.

2.2. Authentication of origin of salmon by analysis of fatty acid composition

Total lipids were extracted from fish samples with 2:1 (v/v) chloroform: methanol and washed with 0.88% KCl (Folch, Lees, & Sloane Stanley, 1957). Fatty acid methyl esters were prepared using 5% hydrochloric acid in methanol (Stoffel, Chu, & Ahrens, 1959), heated overnight at 76 °C and extracted into hexane. Analysis of fatty acid methyl esters was completed by gas chromatography using a 30-m Omegawax TM 320 fused silica capillary column (Supelco, Bellefonte, PA). Oven temperature was initiated at 175 °C and increased 3 °C/min to 220 °C. Flow rate of the carrier gas helium was 30 mL/min. Retention times of samples were compared to standards of fatty acid methyl esters (Matreya, LLC, Pleasant Gap, PA, Supelco, Bellefonte, PA, and Nu-Check Prep Inc., Elysian, MN). Fatty acids are reported as percent of total identified (Belury & Kempa-Steczko, 1997).

2.3. Preparation of salmon

Salmon fillets were washed with tap water and placed on paper towels to remove excess water. Portions (58–75 g) of each were either pan-fried (350–375 °C, 7 min), steamed (212 °C, 10 min), baked (350 °C, 45 min) or broiled (375 °C, 10 min). No ingredients were added prior to cooking. Skinless portions of uncooked and cooked salmon were homogenized (Cuisinart Model CBT-500, Shelton, CT). Aliquots were removed to determine moisture content. The remainder of homogenate was transferred to 50 mL polypropylene test tubes, blanketed with nitrogen gas prior to sealing and stored at −80 °C for a maximum of two weeks before determining Ast content and digested in vitro.