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Chemical stability of astaxanthin nanodispersions in orange juice and skimmed milk as model food systems

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ABSTRACT

Solubilising astaxanthin in nanodispersion systems is a promising approach to incorporate astaxanthin into water-based food formulations. In this research, the chemical stabilities of astaxanthin nanodispersions diluted in orange juice and skimmed milk as model food systems and in deionised water as a control were evaluated. The nanodispersions displayed significantly ($p < 0.05$) better stability in food systems compared to the control. The effects of stabilisers and dilution factor were also studied. In skimmed milk and deionised water, the type of stabiliser had a significant effect ($p < 0.05$) on astaxanthin degradation during storage. *In vitro* cellular uptake of astaxanthin from diluted astaxanthin nanodispersions in selected food systems was also evaluated. The cellular uptake of astaxanthin nanodispersions in skimmed milk was significantly higher ($p < 0.05$) than that of astaxanthin nanodispersions in orange juice and deionised water. High *in vitro* cellular uptake of astaxanthin from the prepared astaxanthin nanodispersions can be achieved via incorporation into protein-based foods such as milk.

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1. Introduction

Functional foods are food products (such as beverages, soups, and spreads) that contain health-promoting active compounds such as carotenoids (Ribeiro, Schuchmann, Engel, Walz, & Briviba, 2010; Tan & Nakajima, 2005). Due to their highly unsaturated structures, carotenoids such as astaxanthin are very sensitive to heat, oxygen and light, any one of which may produce damaging oxidising agents such as free radicals (Higuera-Ciapara, Felix-Valenzuela, & Goycoolea, 2006). For maximal efficacy, carotenoids should remain stable when added to different food formulations, and thus nanodispersion systems seem to be useful in many food and pharmaceutical applications because of their high stability, water solubility and high bioavailability, in addition to their ease of processing (Ribeiro, Ax, & Schubert, 2003). Therefore, astaxanthin nanodispersions have been produced and optimised (Anarjan, Mirhosseini, Baharin, & Tan, 2010; Anarjan, Tan, Nehdi, & Ling, 2012; Anarjan et al., 2011). Subsequently, in this study, the bioavailability and stability of astaxanthin nanodispersions was investigated after dilution in selected food products. Skimmed milk and orange juice were selected as model food systems, with deionised water as a control. The orange juice and skimmed milk were selected as two common food systems with distinct characteristics. Orange juice was selected because of its low pH, the desirable application of produced nanodispersions as a colorant in this system and the presence of active ingredients such as sugar and ascorbic acid. Skimmed milk was selected because it can be used as a medium for astaxanthin enrichment and because skimmed milk is also a good medium to study the effect of milk ingredients, such as various proteins and metal ions, on prepared nanodispersions.

In vitro methods for bioavailability studies such as cellular uptake measurements are preferred to human or animal models due to their potential to provide useful insights about the relative bioavailability of compounds and the effects of different parameters on the bioaccessibility of ingested nutrients. *In vitro* models are also quite inexpensive and technically simple and can be used to screen numerous samples (Failla & Chitchumroonchokchai, 2005; Ribeiro et al., 2006). Generally, the human colon carcinoma-derived cell line HT-29 has been used as a more relevant *in vitro* model for the investigation of intestinal absorption, and this cell line was also employed in this study for bioavailability measurements of astaxanthin nanodispersions in selected food systems.

The nanodispersions were prepared according to an emulsification-diffusion technique. Polysorbate 20 (PS20), sodium caseinate (SC), and gum Arabic (GA), individually and in combination with optimised proportions (OPT), were used as stabiliser systems in the preparation of astaxanthin nanodispersions.

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2. Materials and methods

2.1. Materials

Astaxanthin (>90%) was purchased from Kailu Ever Brilliance Biotechnology Co., Ltd. (Beijing, China). Streptomycin, penicillin, foetal bovine serum (FBS) and trypsin 0.25% were obtained from GIBCO (Grand Island, USA). HT-29 cells (ATCC No. HTB 38, a human colon carcinoma cell line) and modified McCoy's 5a medium (ATCC 30-2007) were purchased from the American Type Culture Collection (ATCC, MD, USA). Phosphate-buffered saline (PBS) and gum Arabic (GA) were donated by Sigma (MO, USA) and Merck Co. (Darmstadt, Germany), respectively. HPLC- and analytical-grade dichloromethane, acetone, acetonitrile, methanol, sodium azide, phosphate buffer (pH 7), polysorbate 20 (PS20) and sodium caseinate (SC) were acquired from Fisher Scientific (Leicestershire, UK). Orange juice concentrate (commercial brand of 'Sunquick') and skimmed milk powder (commercial brand of 'Sunluc') were purchased from a local supermarket (Serdang, Malaysia). Orange juice concentrate (50 °Brix, total protein = 0.6 g/100 mL, total carbohydrate = 7.6 g/100 mL, total sugar = 7.5 g/100 mL, total vitamin C = 9 mg/100 mL, density = 1.3 g/mL) and skimmed milk powder (protein = 3.75 g/100 mL, lactose = 5.1 g/100 mL, ash = 7.8% w/w, fat < 1 g/100 mL) were diluted 1 g_{juice}:9 g_{water} and 25 g_{powder}:250 g_{water}, correspondingly.

2.2. Preparation of astaxanthin nanodispersions

Selected stabilisers (2.5% w/w) were dissolved in 0.05 M phosphate buffer (pH 7) at 40 °C containing 0.02% w/w sodium azide under magnetic stirring overnight and centrifuged at 3000 rpm for 5 min in a KOBOTA 2010 centrifuge (Tokyo, Japan). Astaxanthin (0.08% w/w) was dissolved in a mixture of 62% w/w acetone and 38% w/w dichloromethane, added to the aqueous phase at a ratio of 11.5% w/w organic/aqueous phase, and homogenised with a conventional homogeniser (Silverson, L4R, Buckinghamshire, UK) at 5000 rpm for 5 min. Subsequently, the mixture was passed through a high-pressure homogeniser (APV, Crawley, UK) three times at 30 MPa (Anarjan et al., 2011). The solvent was then removed from the produced nanoemulsion by rotary evaporation (Eyela NE-1001, Tokya Rikakikai Co., Ltd., Tokyo, Japan) at 150 Pa, 25 °C and 100 rpm to convert the astaxanthin nanoemulsions into astaxanthin nanodispersions. Astaxanthin nanodispersions were prepared with selected stabilisers, namely, PS20, SC and GA, individually as well as an optimised blend (29% w/w PS20, 65% w/w SC and 6% w/w GA, OPT) (Anarjan et al., 2012).

The samples were diluted in skimmed milk, orange juice and deionised water to different total astaxanthin concentrations according to the following dilution equation:

$$C_1 \times V_1 = C_2 \times (V_2 + V_1) \quad (1)$$

where C_1 is the initial concentration of astaxanthin in the nanodispersions (Table 1), V_1 is the volume of nanodispersions that should be added to a selected food system with the volume of V_2, and C_2 is the total concentration of astaxanthin in the selected food systems (5, 10, 50 or 100 mg/L).

For each analysis, glasses were filled, tightly sealed and placed in the dark at 5 °C for 4 weeks of storage, and the astaxanthin concentration was quantified periodically.

2.3. Analytical methods

2.3.1. Determination of astaxanthin content

A sample aliquot of 0.5 mL was added to 2 mL of a mixture of dichloromethane and methanol (50:50 v/v) in an amber vial with a screw top. The vial was closed tightly, agitated for 15 min and centrifuged in a KOBOTA 2010 (Tokyo, Japan) centrifuge for 5 min at 800*g*. The extract was then decanted and this extraction process was repeated two more times. The volume of sample was brought up to 10 mL by the addition of methanol. A sample aliquot was filtered with a membrane filter and 40 μL of filtrate was injected into an HPLC system. HPLC analysis was performed with an Agilent liquid chromatography system (Agilent Technologies 1200 Series, Waldbroon, Germany) equipped with a G13150 Diode Array Detector and a Nova-Pak® C18 (3.9 × 300 mm) Waters HPLC column with an isocratic mobile phase consisting of 85% v/v methanol, 5% v/v DCM, 5% v/v acetonitrile and 5% v/v water. Detection was performed at 480 nm (Anarjan et al., 2011, 2012).

2.3.2. Astaxanthin uptakes

The human colon carcinoma HT-29 cell line was selected as a model for human colon epithelial cells. Cells were maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C in supplemented McCoy's 5a medium (with L-glutamine and sodium bicarbonate) containing 10% (v/v) FBS and 1% (v/v) each of streptomycin and penicillin. Three days after seeding, the cells were washed with PBS and incubated with cell culture medium supplemented with the studied diluted astaxanthin nanodispersions with selected food systems. The total concentration of astaxanthin in the food systems was set at 10 μM. Cell monolayers were rinsed with PBS and detached by trypsinisation after an additional 48 h of incubation with the supplemented culture medium under maintenance conditions (37 °C and 95% air, 5% CO_2) and were resuspended in 10 ml culture media. An aliquot of the suspension was used for the determination of cell number, and the remainder of the cell suspension was rinsed with PBS and suspended in 2 ml water/ethanol (1:1, v/v). The cellular astaxanthin was extracted three times with methanol/DCM (1:1, v/v) and quantified by HPLC (Anarjan et al., 2011).

2.3.3. Statistical analysis

To investigate the effects of food product, stabiliser type and dilution factor, linear regression analysis was used to determine the degradation rate constants for astaxanthin under all studied conditions. One-way analysis of variance was performed to study the effect of selected food systems on *in vitro* cellular uptake of astaxanthin in nanodispersions. Significant differences ($p < 0.05$) between means were evaluated by Tukey's multiple-range tests.

Table 1
Particle size, polydispersity index and astaxanthin loss (% w/w) of astaxanthin nanodispersions prepared using different stabiliser systems (Anarjan et al., 2012).

Characteristics	Stabiliser type[1]			
	OPT	SC	PS20	GA
Particle size (nm)	98.3 ± 4.27[a]	113.3 ± 5.58[b]	111.2 ± 3.9[b]	156.8 ± 4.9[c]
PDI	0.348 ± 0.059[bc]	0.411 ± 0.048[b]	0.482 ± 0.075[ab]	0.303 ± 0.035[c]
Astaxanthin loss (% w/w)	14.0 ± 3.1[c]	12.4 ± 4.0[c]	26.7 ± 2.9[b]	41.3 ± 6.6[a]

Values are means ± standard deviations (n = 6).
[a–c] Different letters show statistically significant differences between each row values ($p < 0.05$), comparison tests were done between the values of each response.
[1] SC: sodium caseinate, PS20: polysorbate 20, GA: gum Arabic, OPT: optimum formulated three component stabiliser system (29% w/w PS20, 65% w/w SC and 6% w/w GA).

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