Recent advances in technologies for vitamin A protection in foods

S. M. Loveday s.loveday@massey.ac.nz

H. Singh* h.singh@massey.ac.nz

Riddet Institute
Massey University
Private Bag 11 222
Palmerston North
NEW ZEALAND

* Corresponding author.

Tel.: +64 6 350 4401
Fax: +64 6 350 5655
Abstract

Vitamin A deficiency affects many children in the developing world, and is preventable via food or pharmaceutical supplementation. The main technical barrier to the fortification of food with vitamin A is its susceptibility to oxidation and isomerization, which result in loss of nutritional efficacy. This review discusses recent technological avenues for stabilizing vitamin A in foods.

Key Words

vitamin A, retinoids, encapsulation, nutrition, nutraceutical, food fortification, oxidation, isomerization, nanotechnology

Introduction

‘Vitamin A’ refers to a group of polyunsaturated hydrocarbons with important nutritional roles in humans. The main compounds in this group are the retinoids, which are chemical derivatives of retinol (Fig. 1), and provitamin A carotenoids, which are partially converted to retinoids in vivo.

Adequate intake of vitamin A is vital to childhood development because of its role in vision and ocular health, immune system development, and neurological function. Vitamin A deficiency is a major cause of death and disease in the developing world, especially among mothers and infants.. Supplementation with large, pharmaceutically administered doses of vitamin A can substantially reduce the incidence and severity of some infectious diseases (Villamor & Fawzi, 2005).
Fortification of foods is another strategy for combating vitamin A deficiency, but fortification is not straightforward, for several reasons. Firstly, as vitamin A is accumulated in the adipose tissue and high levels are acutely toxic, the fortification of processed foods must be regulated to avoid excessive vitamin A intake (Dary & Mora, 2002). Secondly, vitamin A is poorly dispersible in aqueous systems such as beverages and high moisture foods. Finally, vitamin A is highly labile under ambient conditions, a problem that affects both food and pharmaceutical supplementation routes. Large losses of vitamin A activity can occur during processing, transportation and storage of fortified foods (Dary & Mora, 2002). Adding vitamin A above the intended fortification level to compensate for losses is undesirable because of the potential for overdosing.

Technologies that enhance the stability of vitamin A in foods are required for ensuring the safety and efficacy of the vitamin A fortification of foods. This paper briefly discusses the factors affecting vitamin A stability and then discusses the present state of the art in vitamin A delivery technologies.

Chemical instability of vitamin A

The conjugated double bond system of retinoids (Fig. 1) and carotenoids presents an electron-dense region that is attractive to electron-deficient species, especially radicals. Retinoid degradation displays characteristics that are typical of radical reactions – catalysis by light, transition metals and free-radical-producing substances, and inhibition by free-radical-quenching chemicals. Principles developed
in the study of polyunsaturated lipid reactions are relevant to retinoid chemistry because of the common polyene chain (see Frankel, 2005). The oxidation pathways for retinoids have been discussed in a number of reviews, e.g. El-Agamey et al. (2004).

The double bonds in the polyene chain of retinoids can undergo cis–trans isomerization, especially at positions 9, 11 and 13. All-trans retinoids are predominant in food but minor amounts of other isomers may also be present (Brinkmann, Dehne, Oei, Tiebach, & Baltes, 1995). All-trans retinol has maximal vitamin A activity, but isomerization during the processing and storage of food (Ball, 1998) results in partial loss of activity (Table 1). Several reaction schemes for geometric isomerization have been proposed, e.g. Rozanowska et al. (2005), but mechanistic understanding of isomerization reactions is limited. Heat-induced isomerization of retinoids produces mainly 13-cis isomers in milk (Panfili, Manzi, & Pizzoferrato, 1998).

Oxygen accelerates photo-catalysed degradation of retinoids under some circumstances, but degradation in the presence of oxygen is relatively slow without a catalyst such as light or chemically generated free radicals (Failloux, Bonnet, Perrier, & Baron, 2004).

Degradation of vitamin A in foods is accelerated by exposure to light, especially ultraviolet light at wavelengths below 415 nm (Garcia-Fuentes, Torres, & Alonso, 2003). Retinoids are degraded faster under ultraviolet-A light (UV-A, 315–400 nm) than under ultraviolet-B light (UV-B, 280–315 nm) (Failloux et al., 2004).
Technologies for stabilizing retinoids

The degradation of retinoids in aqueous solution is rapid (Semenova, Cooper, Wilson, & Converse, 2002), and the solubility of retinoids in aqueous solvents is poor because of their low polarity. Dispersibility and stability can be improved by incorporating retinoids into colloidal carrier particles. Carriers include single and double emulsions, liposomes, solid lipid nanoparticles and polymeric micro- or nanoparticles. Complexing retinoids with molecular carriers, such as cyclodextrins and proteins, can also improve their stability.

Many of these technologies have been developed for cosmetic or pharmaceutical applications and their performance in food systems is poorly characterized. For example, the degradation of stabilized retinoids is often evaluated in model cosmetic emulsions or gels.

The efficiency with which an active agent (AA) is incorporated into a carrier matrix is expressed as a percentage, referred to as the entrapment efficiency:

\[
\text{entrapment efficiency} = \frac{\text{total AA} - \text{unentrapped AA}}{\text{total AA}} \times 100
\]

The capacity of an encapsulation system to carry an AA, or ‘loading capacity’, is expressed as the percentage by weight of the ‘capsule’ phase (entrapped AA + matrix) that comprises AA:

\[
\text{loading capacity} = \frac{\text{entrapped AA}}{\text{entrapped AA} + \text{matrix}} \times 100
\]
1. Emulsion systems

Retinoids can be incorporated into foods as emulsions using an oil-based carrier. The choice of carrier material is important because the oxidative stability of the incorporated retinoid is influenced by droplet physical characteristics (e.g. solid/liquid state, interfacial layer thickness) and chemical characteristics (e.g. degree of unsaturation, fatty acid chain length, presence of natural antioxidants). The basic principles of emulsion preparation and stabilization have recently been reviewed (McClements, Decker, & Weiss, 2007) and are not discussed here.

Surprisingly few applications of oil-in-water (O/W) emulsions to encapsulate retinoids are detailed in the scientific literature. A model cosmetic vehicle was developed in the work of Carlotti, Rossatto, and Gallarate (2002), in which vitamin A palmitate was dissolved in octyl octanoate and emulsified in water with Montanov 68 EC® (cetearyl alcohol and cetearyl glucoside). Vitamin A palmitate degraded slightly faster in an O/W emulsion than in bulk octyl octanoate, which was attributed to localization of vitamin A palmitate at the oil–water interface (Carlotti et al., 2002).

Several patents for the use of O/W emulsions to deliver fat-soluble vitamins into foods or pharmaceuticals have been filed (Hähnlein, Hanse, & Olesen, 1998; Yaghmur et al., 2007).

Microemulsions are thermodynamically stable mixtures of water, oil and one or more amphiphiles, which assemble spontaneously into
nanometre-scale droplets (Flanagan & Singh, 2006). Suitable food-grade surfactants include ethoxylated mono- and diacylglycerides and phospholipids. Ethanol may be required as a co-surfactant to solubilize long chain triglycerides (Flanagan, Kortegaard, Pinder, Rades, & Singh, 2006).

O/W microemulsions are an efficient vehicle for incorporating hydrophobic nutrients into aqueous systems, e.g. lycopene (Garti, Yaghmur, Aserin, Spernath, Elfakess, & Ezrahi, 2004) and β-carotene (Szymula, 2004). On exposure to sunlight, lycopene degrades more slowly in O/W microemulsions than in an organic solvent (Garti et al., 2004). However, Szymula (2004) reported that β-carotene degradation in sunlight was fastest in O/W microemulsions, followed by water-in-oil (W/O) microemulsions and pure pentanol. Szymula (2004) suggested that the high concentration of β-carotene in oil droplets of the O/W microemulsion promoted degradation. Similar results have been reported with retinol in liposomes (Tesoriere, Darpa, Re, & Livrea, 1997).

A patent for the use of O/W or W/O microemulsions to deliver nutraceutical ingredients in foods has been granted (Garti, Abraham, Spernath, & Idit, 2007), and the technology is marketed by NutraLease Ltd (http://www.nutralease.com).

A double emulsion comprises either oil droplets inside water droplets, suspended in an oil-based continuous phase (oil-in-water-in-oil, O/W/O), or water droplets inside oil droplets in an aqueous continuous phase (water-in-oil-in-water, W/O/W).
Yoshida, Sekine, Matsuzaki, Yanaki, and Yamaguchi (1999) made O/W/O emulsions in which the outer oil phase contained an organophilic clay and a non-ionic surfactant. Retinol was incorporated into different types of emulsions, and stability decreased in the order O/W/O > W/O > O/W. Yoshida et al. (1999) attributed the stabilizing effect of the O/W/O emulsion to the exclusion of oxygen from the inner oil phase by a surrounding water layer. Retinol in the outer continuous phase of the W/O emulsion was more stable than retinol in the disperse phase of the O/W emulsion, which does not seem to be consistent with this theory. Retinol stability was thought to be inadvertently compromised by lipid peroxide impurities in the surfactant and metallic impurities in the clay, and stability was improved by both water-soluble and oil-soluble antioxidants (Yoshida et al., 1999).

2. Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) with diameter 50–1000 nm have been used for the delivery of lipophilic drugs and cosmetics because they are well tolerated by the body (Müller, Mäder, & Gohla, 2000). The carrier particles are made from lipids that solidify at room temperature to form a crystalline or amorphous undercooled matrix in which the AA is incorporated. To prepare SLNs, the AA (in this case retinol, retinoic acid or a retinol ester such as retinyl palmitate) is first solubilized in melted lipid, forming a ‘melt’, and nanoparticles are prepared from the melt in one of three ways (Müller et al., 2000).
• Hot homogenization: the melt is dispersed in a hot aqueous solution of surfactant, homogenized at high pressure and then cooled to room temperature.

• Cold homogenization: the melt is cooled to room temperature and ground to microparticles. These are dispersed in cold surfactant solution and homogenized, which produces cavitation forces that are sufficient to break microparticles into nanoparticles.

• Microemulsion technique: the melt is dispersed in hot surfactant solution to generate a microemulsion, which is then added to a cold aqueous medium, causing solid lipid particles to precipitate.

Patents have been granted for the manufacture of SLNs by hot or cold homogenization (Lucks & Müller, 1996) and by the microemulsion technique (Gasco, 2002). Retinoid-loaded SLNs have been produced by hot homogenization (Carlotti, Sapino, Trotta, Battaglia, Vione, & Pelizzetti, 2005; Jenning & Gohla, 2000; Lim, Lee, & Kim, 2004) and the microemulsion technique (Carlotti et al., 2005).

Three models of drug incorporation into the SLN matrix have been proposed: a homogeneous matrix, the outer shell enriched with AA and the inner core enriched with AA (Müller et al., 2000). The three models are illustrated and discussed in more detail by McClements et al. (2007).

A number of parameters affect the internal and membrane structure of SLNs, their stability against aggregation and the protection imparted to an entrapped AA. Cortesi, Esposito, Luca, and Nastruzzi
(2002) investigated the effect of numerous processing parameters on SLN size, recovery and morphology.

**Manufacture method**

Cold homogenization favours a homogeneous matrix. In hot homogenization methods, a shell rich in AA is formed if the lipid crystallizes before the AA. If the AA crystallizes at a lower temperature than the matrix, an enriched core forms. All-*trans* retinol melts at 62–64°C (Schwieter & Isler, 1971).

**Surfactant system**

Lim and Kim (2002) reported that the incorporation of all-*trans* retinoic acid into tricaprin SLNs was improved at higher surfactant levels (which also produced smaller particles). They took this to indicate that retinoic acid was incorporated into the surfactant layer rather than into the lipid matrix. At constant surfactant level and composition, retinol stability improved with decreasing particle size and increasing total surface area (Müller, Radtke, & Wissing, 2002), in agreement with the findings of Lim and Kim (2002).

The stability of SLNs against aggregation is affected by the ionic strength of the continuous phase and the charge density at the lipid–water interface (Garcia-Fuentes et al., 2003; Lim & Kim, 2002). A large zeta potential (positive or negative) helps to stabilize SLNs against aggregation, but non-electrostatic effects such as steric stabilization may also be important (Garcia-Fuentes et al., 2003).
Lim and Kim (2002) reported that the aggregation stability of SLNs loaded with all-trans retinoic acid could be optimized by altering the balance of surfactants. Stability was further improved by the inclusion of distearoylphosphatidyl ethanolamine–N-poly(ethylene glycol) (DSPE–PEG), a polymer with both steric- and electrostatic-stabilizing effects. In freeze-dried SLNs, retention of all-trans retinoic acid was > 90% after 3 months of storage at 4°C (Lim et al., 2004).

**Lipid type**

The loading capacities of SLNs are limited by expulsion of the AA from lipid matrices as they crystallize (Müller et al., 2000). Jenning and Gohla (2000) entrapped retinoids in a range of glycerides and waxes. Retention of the AA within the lipid matrix was poor with the waxes and tripalmitate, the lipids forming relatively pure crystals.

Mixed lipids form less perfect crystals, and the AA can be retained in the space created by crystal imperfections (Müller et al., 2000). This type of SLN is sometimes termed a ‘nanostructured lipid carrier’ (NLC) or ‘oil-loaded SLN’. Three types of structures are formed on cooling the lipid mixtures: imperfect crystals containing many imperfections, amorphous undercooled lipid and liquid lipid droplets in a solid lipid matrix (McClements et al., 2007).

Jores, Haberland, Wartewig, Mäder, and Mehnert (2005) found evidence that solid lipids in mixed-lipid SLNs crystallized in a platelet shape, expelling liquid lipid droplets to the surface. Liquid droplets containing a lipophilic marker were poorly protected from the external aqueous environment (Jores et al., 2005). However, Garcia-Fuentes,
Alonso, and Torres (2005) produced tripalmitin SLNs with a short chain triacylglycerol that appeared to form oily domains within SLNs.

In the work of Jenning and Gohla (2001), the stability of retinol in glycercyl behenate SLNs was improved by adding a low melting medium chain triglyceride. Jenning and Gohla (2001) also reported that the stability of different retinoids in mixed-lipid SLNs followed the order of polarity: retinyl palmitate > retinol > retinoic acid.

Hu, Jiang, Du, Yuan, Ye, and Zeng (2005) produced nanoparticles from a mixture of stearic and oleic acids, and reported that increasing oleic acid content produced smoother particles with decreased crystallinity and increased drug loading capacity. Jenning and Gohla (2001) reported that adding 5–10% liquid lipid to SLNs improved the retinol loading capacity and stability, and postulated the existence of liquid and solid lipid domains within particles.

Carlotti et al. (2005) and Sapino, Carlotti, Pelizzetti, Vione, Trotta, and Battaglia (2005) tested the stability of retinyl palmitate in cetyl palmitate, glycercyl behenate and palmitic acid SLNs during exposure to UV light or prolonged storage. Stability was evaluated in model cosmetic systems – an O/W emulsion (Carlotti et al., 2005) and a hydroxyethylcellulose gel (Sapino et al., 2005). Retinyl palmitate in SLNs degraded more slowly than free retinyl palmitate. SLNs made with different lipids had similar light-scattering properties but the least polar lipid, cetyl palmitate, best protected retinyl palmitate against UV irradiation. This may have been due to improved retention of retinyl palmitate in the lipid matrix.
Mixed-lipid nanoparticle carriers have apparently not been patented.

3. Liposomes

Liposomes can be made by adding buffer to powdered phospholipid or a dried phospholipid film formed by rotary evaporation of an organic solvent. The organic solvents used in film formation are not acceptable in food.

Entrapment of retinoids in the lipid bilayers of multilamellar liposomes has been shown to improve their stability in the presence of oxygen (Lee, Yuk, Lee, Lee, Hwang, & Ludescher, 2002). Incorporation of retinol into liposomes at a retinol:phospholipid ratio of 1:100 is up to 99% efficient, but liposomes offer little protection in acidic conditions or at temperatures at or above ambient (Lee et al., 2002). The process used to manufacture liposomes in this case used chloroform and methanol (Lee et al., 2002). Retinol shows greater affinity than retinyl palmitate for entrapment into liposomes (Singh & Das, 1998).

Young and Gregoriadis (1996) reported that incorporating retinol into liposomes accelerated photodegradation relative to free retinol in methanol. Similarly, Tesoriere et al. (1997) found an increase in the retinol degradation rate with increasing concentration of liposome-encapsulated retinol. It was suggested that degradation reactions involving two retinol molecules were accelerated by concentrating retinol in liposomes (Young & Gregoriadis, 1996).

Liposome encapsulation can be combined with other mechanisms to enhance stability. Loukas, Jayasekera, and Gregoriadis (1995)
reported that the rate of riboflavin degradation under UV light was reduced up to 75% by incorporation into liposomes. Complexing riboflavin with $\gamma$-cyclodextrin slowed degradation by a further 80% at the expense of an approximately 50% reduction in entrapment efficiency (Loukas, Jayasekera, & Gregoriadis, 1995). McCormack and Gregoriadis (1998) reported 19% efficient entrapment of a retinol–hydroxypropyl $\beta$-cyclodextrin complex in liposomes but did not examine the effect on retinol oxidation or isomerization.

Liposomes can be stabilized against aggregation by coating with chitosan and incorporating them into microparticles (Ruan, Ng, & Feng, 2004). They can also encapsulate other particles, e.g. polymeric vesicles of palmitoyl glycol chitosan (McPhail, Tetley, Dufes, & Uchegbu, 2000).

In a related technology, lipid-soluble compounds can be incorporated into vesicles of a non-ionic surfactant (niosomes). Encapsulation of $\beta$-carotene in niosomes comprising cholesterol and Tween or Span surfactants slows its degradation in sunlight and hydrogen peroxide solution, relative to free $\beta$-carotene (Palozza, Muzzalupo, Trombino, Valdannini, & Picci, 2006). Manconi, Valenti, Sinico, Lai, Loy, and Fadda (2003) tested a range of non-ionic surfactants for their ability to stabilize all-trans retinoic acid in niosomes irradiated with UV light, and found that two of the formulations improved stability relative to retinoic acid in methanol. However these authors used chloroform to manufacture niosomes, and for that reason the process is not suitable for food applications.
4. Cochleates

Cochleates are micro- or nanoparticles consisting of phospholipid bilayers stacked as sheets and rolled into a spiral configuration with aqueous solutions of multivalent cations between each sheet (BioDelivery Sciences International, 2008). This is illustrated schematically in Fig. 2. Cochleates are prepared by slowly introducing polyvalent cations into anionic liposome suspensions, causing the liposomes to fuse.

In the ‘trapping’ method, calcium chloride is added dropwise to a liposome suspension, and cochleate formation is indicated by an immediate increase in turbidity (Evans & Zasadzinski, 2003). Small unilamellar liposomes prepared by film hydration give more uniform cigar-shaped cochleates than multilamellar liposomes from powdered phospholipids (Zarif, 2005).

For the ‘hydrogel process’, liposomes are mixed with a polymer such as dextran and injected into a solution of a second, non-miscible polymer, e.g. PEG. Calcium is added to the water-in-water emulsion and diffuses slowly from the PEG continuous phase into the dispersed dextran–liposome phase, producing nanocochleates (Santangelo et al., 2000). Sub-micrometre cochleates can be produced with this method whereas the trapping method gives larger cochleates.

Cochleates are most frequently made with dioleoyl phosphatidyl serine and calcium chloride – natural ingredients that are acceptable in food systems (Evans & Zasadzinski, 2003; Walker, Kennedy, &
Zasadzinski, 1997). Calcium can be replaced with zinc ions (Zarif, Jin, Segarra, & Mannino, 2005) or organic cations (Jin, 2004).

Substances in the interior of liposomes are encapsulated in the cochleate when the cation is added. Santangelo et al. (2000) prepared cochleates from liposomes containing amphotericin B, a hydrophobic antimycotic drug. They used the hydrogel method, which gave cochleates with mean diameter 407 nm. The cochleate-encapsulated drug was highly effective against fungal infections in mice (Santangelo et al., 2000). It appears that encapsulating retinoids in cochleates had not been attempted at the time of writing.

Cochleates can be used as intermediates for encapsulating small liposomes or colloidal particles, thus double-encapsulating labile substances (Evans & Zasadzinski, 2003; Walker et al., 1997). Cations are chelated when EDTA is added to a suspension of colloidal particles and cochleates, causing the cochleates to unroll and close into vesicles around the particles. Walker et al. (1997) attached colloidal particles to cochleate phospholipids with specific ligand-receptor molecules, but the encapsulation efficiency was poor.

The company BioDelivery Sciences International Inc. has filed several US and international patents related to the incorporation of vitamin A in nanocochleates to enhance stability. Cochleate manufacture with the hydrogel method is claimed (Mannino & Krause-Elsmore, 2004), as is incorporation of liposomes into cochleates (Krause-Elsmore & Mannino, 2005) and several other applications (Zarif et al., 2005).
5. Cyclodextrin inclusion complexes

Cyclodextrins (CDs) are rings of α-1,4 bonded glucose molecules with a slightly hydrophobic interior that can entrap molecules less polar than water (Szejtli, 1998). Natural, underivatized CDs contain six, seven or eight glucose molecules and are referred to as α-, β- and γ-CDs respectively. Methods for forming complexes between CDs and nutritional or pharmaceutical AAs have been reviewed by Szente (1996).

Retinoid–CD complexes have been prepared by mixing solutions at room temperature in aqueous ethanol (Semenova et al., 2002) or methanol (Guo, Ren, Fang, & Liu, 1995). Another method is to form a film of retinoid on the surface of a flask, add aqueous CD solution to the flask and stir for several days (McCormack & Gregoriadis, 1998; Munoz-Botella, Martin, Del Castillo, Lerner, & Menendez, 2002).

Inclusion of unstable or poorly water-soluble molecules in CDs can improve their solubility in aqueous systems. For example, the aqueous solubility of all-trans retinoic acid increases by more than 100 times after complexation with β-CD (Qi & Shieh, 2002) and more than 10 000 times after complexation with hydroxypropyl β-CD (Lin, Chean, Ng, Chan, & Ho, 2000). The solubility of the β-CD–retinoic acid complex is better at neutral pH than acidic pH (Lin et al., 2000; Yap, Liu, Thenmozhiyal, & Ho, 2005). This may be because COO− groups of retinoic acid molecules, which are more ionized at neutral pH, can interact with the hydroxyl groups of CDs (Lin et al., 2000).
The solubility of β-CD complexes can be improved by organic salts. Qi and Shieh (2002) reported a 26-fold increase in β-CD–retinoic acid complex with the addition of 1.5% sodium acetate. Sodium acetate also improves the solubility of hydrocortisone in β-CD, with an optimum concentration of 2% (Loftsson, Matthiasson, & Masson, 2003). Some organic polymers enhance the solubilizing effect of CDs, e.g. carboxymethylcellulose (Qi & Shieh, 2002) and poly(vinyl pyrrolidone) (Loftsson & Brewster, 1996).

CDs have found widespread application in chromatographic methods and as drug carriers (Szejtli, 1998). Complexation of retinoids with CDs has been demonstrated by several groups (McCormack & Gregoriadis, 1998; Munoz-Botella et al., 2002; Yap et al., 2005). Semenova et al. (2002) reported good encapsulation and protection of all-trans retinol with two cyclodextrin formulations, but did not show sufficient data to verify this.

Association constants for several retinoid-cyclodextrin complexes are shown in Table 2. Binding is most favourable at neutral pH (Lin et al., 2000). Cyclodextrin binding studies have also been carried out on several cis-retinoids (Munoz-Botella et al., 2002; Yap et al., 2005) and carotenoids (Polyakov, Leshina, Konovalova, Hand, & Kispert, 2004).

Complexing retinoids with CDs inhibits photoisomerization (Munoz-Botella et al., 2002) and photodegradation (Yap et al., 2005). The retinoid polyene chain is held in the CD cavity (Yap et al., 2005), which dampens the torsion and rotation required for isomerization
CD complexes of all-trans retinaldehyde photoisomerize preferentially to the 13-cis form but retinoic acid–CD complexes isomerize to a mixture of 9-, 11- and 13-cis isomers (Munoz-Botella et al., 2002).

The molar ratio of CD to retinoid molecule in the inclusion complex is usually 1:1 or 2:1 (Guo et al., 1995; Munoz-Botella et al., 2002), but ratios as high as 4.5:1 have been reported (McCormack & Gregoriadis, 1998). The stoichiometry of the inclusion complex can change as a function of CD concentration, e.g. hydroxypropyl β-CD forms complexes with all-trans retinoic acid in a molar ratio of 1:1 at low CD concentration and in a molar ratio of 2:1 at higher CD concentration (Lin et al., 2000).

CDs are already used in foods to encapsulate colours, flavours and polyunsaturated fatty acids, and to prevent turbidity in drinks.

The stabilizing effect of CDs on vitamin A has been known for some time, and was claimed in a 1955 US patent (Schlenk, Sand, & Tillotson, 1955). A number of Japanese patents have claimed methods for stabilizing vitamins with CD complexes, e.g. Takeshi and Okihiko (1994). In 1994, a US patent for a method of enhancing complexation between lipophilic food additives (including vitamin A) and CDs using natural or synthetic hydrophilic polymers was granted (Loftsson, 1994). Complexes of retinol or retinyl esters with γ-CDs are described in a 1999 US patent (Moldenhauer, Regiert, & Wimmer, 1999).
6. Polymer encapsulation

Polymers used for encapsulating retinoids in solid particles include proteins, polysaccharides and synthetic polymers. The choice of polymer in food systems is restricted by food regulations, and only food-safe solvents may be used in particle manufacture. Encapsulation of lipophilic drugs in polymer particles can be improved by complexation with CDs (Duchene, Ponchel, & Wouessidjewe, 1999).

Hwang, Oh, and Oh (2005) and Lee, Oh, Moon, and Bae (2001) successfully entrapped retinol in 0.5–10 μm silica particles made using a multiple emulsion technique. Encapsulation efficiencies of 7.4–30.9% (Hwang et al., 2005) and 35.9–44.0% (Lee et al., 2001) were achieved. In a similar procedure, Ribeiro, Neufeld, Arnaud, and Chaumeil (1999) produced chitosan-coated alginate microspheres, with mean diameter 500–800 μm, containing soybean oil droplets and an oil-soluble marker dye. The marker dye was released slowly at pH 1.2 and faster at pH 7.5, and the speed of release depended on the thickness of the coating (Ribeiro et al., 1999).

Polymer particles can be prepared by coacervation, which is also referred to as nanoprecipitation, drowning out or solvent displacement. In this technique, the polymer and the AA are dissolved in an organic solvent, which is emulsified in an aqueous surfactant solution. The solvent is evaporated from the O/W emulsion and the polymer precipitates, forming solid particles.
Biodegradable copolymers of lactic acid and glycolic acid (PLGA) have been widely used in drug delivery applications, and at least two research groups have encapsulated retinoids in copolymer microparticles (Cirpanli, Unlu, Calis, & Hincal, 2005; Jeong et al., 2003). Particles of 1–100 μm were prepared with the coacervation technique, and an encapsulation efficiency of up to 74% was reported (Cirpanli et al., 2005; Jeong et al., 2003). However, the solvents commonly used to dissolve PLGA polymers are not permitted in food systems.

Since the 1950s, vitamin A has been encapsulated in solid gelatin beadlets for incorporation into animal feeds. In this process, an emulsion containing antioxidants, starch, gelatin and sugars, is spray congealed and the beadlet is cross linked by thermal or chemical means. Other protein encapsulants include collagen and gliadin.

Gliadin is a hydrophobic wheat protein that is insoluble in water but soluble in 70% ethanol. Hydrophobic AAs can be entrapped in gliadin nanoparticles by coacervation. Gliadin particles of about 500 nm with a payload of 76.4 μg of retinoic acid per milligram of protein were prepared by Duclairoir, Irache, Nakache, Orecchioni, Chabenat, and Popineau (1999). These workers demonstrated controlled release of retinoic acid over 3 h, but did not examine degradation of the entrapped retinoic acid. Preparation of gliadin-coated lipid particles using a salting-out method has also been reported (Mauguet, Legrand, Brujes, Carnelle, Larre, & Popineau, 2002).
Swartschek, Schatton, Müller, and Kreuter (2002) used microparticles of marine sponge collagen (120–300 nm) as carriers for retinol adsorbed on the surface. In a model cosmetic cream containing retinol-loaded collagen particles, retention was over 50% after 8 weeks at 21°C.

It was recently reported that nanoparticle complexes can be formed in mixtures of gum arabic and sodium caseinate, and that the particle characteristics can be tailored by manipulating the ratio of caseinate to gum arabic (Ye, Flanagan, & Singh, 2006). Such protein–carbohydrate nanoparticle complexes may be suitable vehicles for vitamin A encapsulation.

Encapsulation of lipophilic substances in polymer beads has been the subject of several patents. Mandralis and Tuot (1997) patented a process for encapsulating AAs in biopolymer particles by high pressure gelation of a natural polymer encapsulant. Catron and Mann (2000) produced beads loaded with hydrophobic AA by heat setting or chemically cross linking emulsion droplets. Lim and Moss (1983) devised a process in which alginate-containing emulsion droplets are solidified by immersion in alcoholic solutions of multivalent cations. A similar method using a mixture of cationic and anionic polymers is the subject of a patent application (Cattaneo, 2005).
7. Protein binding

In living organisms, retinoids are non-covalently bound by proteins of the ‘lipid-binding protein’ family, which transport fatty acids and other small hydrophobic ligands in plants, animals and bacteria (De Wolf & Brett, 2000). Retinol may also be bound to proteins in food matrices, especially those containing lipid-binding whey proteins, e.g. β-lactoglobulin and bovine serum albumin (BSA).

β-Lactoglobulin exists in bovine milk as a dimer at pH 6.5 with two binding sites per dimer, and has a tertiary structure very similar to those of retinol-binding proteins in the human body (Perez & Calvo, 1995). The fluorescence of retinol is enhanced by β-lactoglobulin, because binding restricts the mobility of the retinol molecule, and fluorescence enhancement can be used to quantify binding affinity (Collini, D’Alfonso, Molinari, Ragona, Catalano, & Baldini, 2003). Retinoic acid dissociates from the retinoic acid–β-lactoglobulin complex below pH 7.0 because of conformational changes in the protein, and will re-associate on neutralization (Zsila, Bikadi, & Simonyi, 2002).

Chemical derivatization of β-lactoglobulin can alter its functional properties, including retinoid binding. Methyl- or ethyl-alkylation of β-lactoglobulin improves the binding of retinol, retinoic acid, retinyl acetate and β-carotene (Dufour & Haertle, 1991). Hattori, Okada, and Takahashi (2000) reported that conjugating carboxymethyl CD to β-lactoglobulin improved the emulsifying activity and pH stability.
Antioxidant properties and retinol binding were not affected (Hattori et al., 2000).

In the work of Shimoyamada, Yoshimura, Tomida, and Watanabe (1996), retinoic acid or retinol complexed with β-lactoglobulin degraded more slowly than the uncomplexed retinoids during 6 h of UV irradiation. Hattori, Watabe, and Takahashi (1995) reported that β-lactoglobulin protected retinol during irradiation and exposure to high temperature or acidic pH. Retinol retention was > 60% after 5 h at 60°C (compared with 30% without β-lactoglobulin); higher temperatures denatured the protein and destroyed the protective effect. More than 80% of the initial retinol was retained after 5 h at 37°C and pH 3–9 (Hattori et al., 1995).

BSA also binds retinoids, resulting in enhanced fluorescence. BSA inhibits light-induced degradation of retinol to a similar extent to β-lactoglobulin, but does not protect retinoic acid (Shimoyamada et al., 1996).

Carotenoids are bound to lipoproteins in some vegetables, cyanobacteria, animals and crustaceans. The structure and the biological function of carotenoproteins have been discussed in the literature (Flower, 1996), and there is potential to use them as means of encapsulating labile nutrients (De Wolf & Brett, 2000).

8. Glassy matrix encapsulation

Encapsulation of β-carotene in amorphous trehalose (a disaccharide) has been shown to slow degradation during extended storage,
provided the trehalose can be prevented from absorbing moisture and crystallizing (Elizalde, Herrera, & Buera, 2002). A process for encapsulating vitamins or minerals in glassy fructo-oligosaccharides has been patented (Leusner, Lakkis, van Lengerich, & Thomas, 2002).

9. Antioxidants

Some antioxidants improve the stability of retinoids during exposure to light or oxygen. Butylated hydroxytoluene (BHT) is an oil-soluble phenolic antioxidant that competes with radical propagation reactions. Carlotti et al. (2002) reported that BHT slowed the degradation of retinyl palmitate under UV irradiation in a sealed container, but Failloux et al. (2004) found no effect in oxygenated retinol solution exposed to UV-A light. Retinoid destruction in oxygenated solutions kept in the dark was slowed by \( \alpha \)-tocopherol but not by \( \beta \)-carotene or ascorbic acid in the work of Ihara, Hashizume, Hirase, and Suzue (1999).

Antioxidants work well as an adjunct to other encapsulation techniques. For example, the retinol stability in O/W/O emulsions is improved by both oil-soluble (BHT) and water-soluble (sodium ascorbate) antioxidants (Yoshida et al., 1999).

Summary and conclusions

Vitamin A supplementation via foods and/or pharmaceuticals has the potential to prevent disease and ensure healthy childhood development in developing countries. Technical barriers to delivering
vitamin A in foods include its poor dispersibility in aqueous systems and its vulnerability to degradation during processing, transport and storage. As the risk of overdosing precludes high dose fortification to compensate for losses, there is a need for technologies that slow vitamin A degradation.

Slowing the degradation of vitamin A relies on protecting it from influences that accelerate degradation, particularly UV light, chemical catalysts (especially metal contaminants and lipid hydroperoxides) and oxygen. Much of the research into technologies for protecting vitamin A does not examine the mechanistic rationale for an improvement in stability, but instead takes a ‘screening’ approach.

Vitamin A supplementation via foods requires that the amount of vitamin A present at the time of consumption is sufficiently high to be therapeutic and sufficiently low to minimise the risk of overdosing. Maintaining vitamin A activity within the bounds of safety and effectiveness requires limiting the loss of activity through oxidation and isomerization. It is therefore important to assess both the loading capacity of an encapsulation system (which depends on entrapment efficiency) and the stability of encapsulated vitamin A under conditions relevant to food processing and storage.

Table 2 summarises the loading capacity, entrapment efficiency and stability improvement of a range of vitamin A protection technologies discussed here. It is evident from table 2 that the entrapment efficiency of encapsulation systems is seldom measured. This is important because a significant proportion of vitamin A mixed with an
encapsulant can remain free in solution or adsorbed on surfaces, where it will be exposed to conditions that catalyse oxidation and/or isomerisation. Without a knowledge of encapsulation efficiency it is impossible to evaluate true loading capacity, and therefore difficult to compare the effectiveness of different systems. It is not possible to evaluate the commercial potential of diverse technologies based on information in the academic literature because the costs of ingredients are never disclosed in articles.

Also notable in Table 2 is a scarcity of technologies that use food-safe manufacturing processes. This probably results from the fact that much vitamin A stabilization research is oriented towards pharmaceutical applications, in which doses are low, or cosmetic products that are applied dermally and not consumed.

Emulsions can act as a vehicle for dispersing large amounts of hydrophobic material in an aqueous system. The photostability of labile nutrients is sometimes improved by emulsification, and synergy between oil- and water-soluble antioxidants is enhanced in these systems. Microemulsions and multiple emulsions have shown some promise in stabilizing hydrophobic nutrients, and warrant further investigation.

SLNs provide a physical barrier between retinoids and oxidation catalysts, and their light-scattering properties (Müller et al., 2002) reduce the intensity of light reaching the AAs entrapped in them. SLNs made with anionic stabilizers tend to aggregate at low pH, but non-ionic polymers can provide sufficient steric stabilization to
overcome this. Amphiphilic polymer stabilizers such as PEG derivatives and copolymers of polyoxyethylene and polyoxypropylene (trade names Pluronic, Poloxamer) provide effective steric stabilization at low pH.

Stabilization in solid lipid particles requires efficient incorporation and retention in the lipid matrix, which is best achieved with mixed lipids that form impure crystals, undercooled melts or oil droplets within a solid matrix. The lipid-soluble antioxidants butylated hydroxyanisole, BHT, propyl gallate and α-tocopherol can further enhance the stability of lipid-encapsulated retinoids.

Entrapment in liposomes protects vitamin A under some conditions, but little protection is afforded at acidic pH and ambient or higher temperature. Some authors have reported accelerated degradation of vitamin A in liposomes, relative to free vitamin A. This has been speculatively attributed to the high local concentration inside liposomes, which presumably accelerates autoxidation.

Methods for preparing liposomes in the laboratory often use solvents that are unacceptable for food systems, and published results may be difficult to reproduce in food-safe systems. Liposomes can be made more robust with a protective coating (Ruan et al., 2004) or by incorporation inside a multilayer encapsulation system (Evans & Zasadzinski, 2003).

Lipid cochleates are very effective at protecting and delivering drugs, but to date there have been no applications in foods, probably
because existing processes for making cochleates use toxic solvents. Cochleates may be useful as intermediates in the preparation of ‘vesicles in vesicles’ (Evans & Zasadzinski, 2003).

Retinoids may be more stable dispersed or dissolved in an aqueous phase than in lipid droplets or particles, for two reasons: (1) oxygen is less soluble in water than in lipids, and (2) lipid hydroperoxides are absent in the aqueous phase. Stability would be compromised if retinoids were brought into close contact with aqueous oxidation catalysts such as transition metal ions. The aqueous solubility of vitamin A is greatly improved by complexing with CDs.

Vitamin A has been incorporated into solid particles of proteins (gelatin, collagen, gliadin), carbohydrates (alginate, chitosan) and synthetic polymers (silicates, PLGA copolymers). Encapsulation processes have been reported and, in some cases, patented, but the stability of polymer-encapsulated vitamin A is not often evaluated in the literature. Polymer particles range in size from hundreds of nanometres to hundreds of micrometres, which may not be suitable for liquid foods.

Carotenoids are often complexed with proteins in biological systems, and vitamin A will form water-soluble complexes with certain proteins. BSA and β-lactoglobulin offer some protection against photo-oxidation, heating and acidic pH. Other plant or animal proteins may also form soluble complexes with retinoids that preserve vitamin A activity in food systems.
It is uncommon to find ‘silver bullet’ solutions to food preservation problems; a combination of approaches such as the ‘hurdle-technology’ concept (Leistner & Gorris, 1995) is more often successful. Protecting vitamin A is just such a problem, because retinoids and carotenoids must be stabilized against several chemical and physical degradation catalysts, and protection is often achieved at the expense of loading capacity. Synergistic protective effects are seen when individual technologies are combined, such as incorporating antioxidants into emulsions or SLNs, and encapsulating CD complexes inside liposomes. In our opinion, the most promising advances in vitamin A protection in foods will come from consideration of the multifaceted nature of vitamin degradation and the potential to combine technologies in ways that take advantage of their individual strengths.
References


heating, oxidation, and irradiation. *Bioscience Biotechnology and Biochemistry, 59*(12), 2295-2297.


Ruan, G., Ng, J. K., & Feng, S. S. (2004). Effects of polymer, organic solvent and mixing strength on integrity of proteins and liposomes
encapsulated in polymeric microspheres fabricated by the double

Santangelo, R., Paderu, P., Delmas, G., Chen, Z. W., Mannino, R.,
mouse model of systemic candidiasis. *Antimicrobial Agents and
Chemotherapy, 44*(9), 2356-2360.

Sapino, S., Carlotti, M. E., Pelizzetti, E., Vione, D., Trotta, M., &
Battaglia, L. (2005). Protective effect of SLNs encapsulation on the
photodegradation and thermal degradation of retinyl palmitate
introduced in hydroxyethylcellulose gel. *Journal of Drug Delivery
Science and Technology, 15*(2), 159-165.

Schlenk, H., Sand, D., & Tillotson, J. (1955). Stabilization of

Gutmann & U. Solms (Eds.), *Carotenoids* (pp. 5-35). Basel, Stuttgart:
Birkhäuser-Verlag.

Semenova, E. M., Cooper, A., Wilson, C. G., & Converse, C. A.
(2002). Stabilization of all-trans-retinol by cyclodextrins: a
comparative study using HPLC and fluorescence spectroscopy.
*Journal of Inclusion Phenomena and Macrocyclic Chemistry, 44*(1-4),
155-158.

Shimoyamada, M., Yoshimura, H., Tomida, K., & Watanabe, K.
(1996). Stabilities of bovine beta-lactoglobulin/retinol or retinoic acid
complexes against tryptic hydrolysis, heating and light-induced


This work was funded by BASF, Germany and the NZ Foundation for Research, Science and Technology. The authors thank BioDelivery Sciences International Inc. for permission to reproduce figure 2.
Fig. 1. Structure of selected retinoids, where R is one of: A, retinol; B, retinoic acid; C, retinaldehyde; D, retinyl palmitate.
Fig. 2. Schematic representation of nanocochleate structure.

Copyright BioDelivery Sciences International, Inc.
Table 1. Relative vitamin A activity of retinyl acetate isomers.

<table>
<thead>
<tr>
<th>RA(^a) isomer</th>
<th>Relative vitamin A activity(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>all-trans</td>
<td>100</td>
</tr>
<tr>
<td>13-cis</td>
<td>75</td>
</tr>
<tr>
<td>11-cis</td>
<td></td>
</tr>
<tr>
<td>9-cis</td>
<td>22</td>
</tr>
<tr>
<td>7-cis</td>
<td></td>
</tr>
<tr>
<td>9,13-di-cis</td>
<td>24</td>
</tr>
<tr>
<td>11,13-di-cis</td>
<td>23</td>
</tr>
</tbody>
</table>

\(^a\)RA, retinyl acetate

\(^b\)the activity of the all-trans isomer is arbitrarily designated as 100%
### Table 2. Ability of various encapsulation systems to entrap and protect vitamin A.

<table>
<thead>
<tr>
<th>reference</th>
<th>technology</th>
<th>food safe?</th>
<th>AA(^a)</th>
<th>loading capacity(^b) % w/w</th>
<th>entrapment efficiency(^c) %</th>
<th>stability improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoshida et al. (1999)</td>
<td>O/W/O emulsion</td>
<td>?</td>
<td>RO</td>
<td>0.47</td>
<td>83.5</td>
<td>after storage at 50°C for 4 weeks, 60% of retinol remained, while retinol in paraffin was completely degraded.</td>
</tr>
<tr>
<td>Jenning &amp; Gohla (2001)</td>
<td>glyceryl behenate SLN</td>
<td>yes</td>
<td>RO</td>
<td>10(^d)</td>
<td>ND(^e)</td>
<td>after storage at 40°C for 160 days, 60% of retinol remained, compared with 50% of retinol in an O/W emulsion</td>
</tr>
<tr>
<td>Carlotti et al. (2005)</td>
<td>cetyl palmitate SLN in cosmetic O/W emulsion</td>
<td>?</td>
<td>RP</td>
<td>9(^d)</td>
<td>ND</td>
<td>after UV irradiation for 120 min, 70% of retinol in SLN remained, compared with 8% of retinol in an O/W emulsion</td>
</tr>
<tr>
<td></td>
<td>glyceryl behenate SLN in cosmetic O/W emulsion</td>
<td>?</td>
<td>RP</td>
<td>9(^d)</td>
<td>ND</td>
<td>after UV irradiation for 120 min, 51% of retinol in SLN remained, compared with 8% of retinol in an O/W emulsion</td>
</tr>
<tr>
<td></td>
<td>palmitic acid SLN in cosmetic O/W emulsion</td>
<td>?</td>
<td>RP</td>
<td>5.6(^d)</td>
<td>ND</td>
<td>after UV irradiation for 120 min, 66% of retinol in SLN remained, compared with 8% of retinol in an O/W emulsion</td>
</tr>
<tr>
<td>Study (Year)</td>
<td>System/Complex Type</td>
<td>Complexed/Free</td>
<td>Lipid/Drug</td>
<td>Stability Parameter</td>
<td>Stability Parameter Units</td>
<td>Comments</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------------------------------------</td>
<td>----------------</td>
<td>------------</td>
<td>--------------------</td>
<td>---------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Lee et al. (2002)</td>
<td>PC and soybean oil liposomes</td>
<td>no</td>
<td>RO</td>
<td>0.99</td>
<td>99.25 ± 0.47</td>
<td>after 8 days at 25°C, pH 7.0 without light, 50% of retinol in liposomes remained, while free retinol in buffer was completely degraded.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munoz-Botella et al.  (2002)</td>
<td>β-cyclodextrin complex</td>
<td>no</td>
<td>ATRA</td>
<td>0.13^d</td>
<td>ND</td>
<td>after exposure to light for 60 min, 44.3% of ATRA with β-CD remained in all-trans form, compared with 31.8% for ATRA in ethanol.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DM-β-cyclodextrin complex</td>
<td>no</td>
<td>ATRA</td>
<td>0.11^d</td>
<td>ND</td>
<td>after exposure to light for 60 min, 54.6% of ATRA with DM-β-CD remained in all-trans form, compared with 31.8% of ATRA in ethanol.</td>
</tr>
<tr>
<td>Lin et al. (2000)</td>
<td>2-HP-β-cyclodextrin complex</td>
<td>yes</td>
<td>ATRA</td>
<td>0.57^d</td>
<td>ND</td>
<td>after exposure to light at 40°C for 6 h, 22% of ATRA with HP-β-CD remained, compared with 18% of ATRA in methanol.</td>
</tr>
<tr>
<td>Lee et al. (2001)</td>
<td>silica microspheres</td>
<td>no</td>
<td>RO</td>
<td>2.23-2.82</td>
<td>35.9-44.0</td>
<td>not tested</td>
</tr>
<tr>
<td>Hwang et al. (2005)</td>
<td>silica microspheres</td>
<td>no</td>
<td>RO</td>
<td>not reported</td>
<td>7.4-30.9</td>
<td>not tested</td>
</tr>
<tr>
<td>Jeong et al. (2003)</td>
<td>polymer particles</td>
<td>no</td>
<td>ATRA</td>
<td>1.66-8.83</td>
<td>27.1-54.9</td>
<td>not tested</td>
</tr>
<tr>
<td>Duclairoir et al.</td>
<td>gliadin nanoparticles</td>
<td>yes</td>
<td>ATRA</td>
<td>7.64</td>
<td>75</td>
<td>not tested</td>
</tr>
<tr>
<td>Study</td>
<td>Complex Type</td>
<td>Presence</td>
<td>RO</td>
<td>pH</td>
<td>ND</td>
<td>Details</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------------------</td>
<td>----------</td>
<td>----</td>
<td>----</td>
<td>-----</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Shimoyamada <em>et al.</em> (1996)</td>
<td>β-lactoglobulin complex</td>
<td>yes</td>
<td>RO</td>
<td>1.3&lt;sup&gt;d,h&lt;/sup&gt;</td>
<td>ND</td>
<td>after 6 h under UV light, 92% of retinol remained, while 45% or free retinol remained.</td>
</tr>
<tr>
<td>Hattori <em>et al.</em> (1995)</td>
<td>β-lactoglobulin complex</td>
<td>yes</td>
<td>RO</td>
<td>0.7&lt;sup&gt;d,h&lt;/sup&gt;</td>
<td>ND</td>
<td>after holding at 50°C for 5 h, 80% of retinol with β-lactoglobulin remained, while 25% of retinol in buffer remained.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>after exposure to fluorescent light for 5 h, 36% of retinol with β-lactoglobulin remained, while 20% of retinol in buffer remained.</td>
</tr>
</tbody>
</table>
active agent: RO, retinol; RP, retinyl palmitate; ATRA, all-trans retinoic acid

loading capacity = \frac{\text{entrapped AA}}{\text{entrapped AA + matrix}} \times 100

entrapment efficiency = \frac{\text{total AA - unentrapped AA}}{\text{total AA}} \times 100

nominal loading capacity, assuming 100% entrapment efficiency

not determined

DM-\beta-cyclodextrin, DM-\beta-CD: heptakis (2,6-O-dimethyl)-beta-cyclodextrin

HP-\beta-cyclodextrin, HP-\beta-CD: hydroxypropyl-beta-cyclodextrin

calculated from concentrations of \beta-lactoglobulin and retinol using the molecular weight of \beta-lactoglobulin-A reported in Farrell et al., 2004
Recent advances in technologies for vitamin A protection in foods.

Loveday, SM

2008