Chapter 8
Encapsulation of Carotenoids

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8.1 Introduction

Carotenoids are natural pigments, which are synthesized by microorganisms and plants. More than 600 naturally occurring carotenoids have been found in the nature. The main sources of carotenoids are fruits, vegetables, leaves, peppers, and certain types of fishes, sea foods, and birds. Carotenoids may protect cells against photosensitization and work as light-absorbing pigments during photosynthesis. Some carotenoids may inhibit the destructive effect of reactive oxygen species. Due to the antioxidative properties of carotenoids, many investigations regarding their protective effects against cardiovascular diseases and certain types of cancers, as well as other degenerative illnesses, have been carried out in the last years (Briviba et al. 2004; Krinsky et al. 2004; Kirsh et al. 2006). A diet rich in carotenoids may also contribute to photoprotection against UV radiation (Stahl et al. 2006). In vitro studies have shown that carotenoids such as β-cryptoxanthin and lycopene stimulate bone formation and mineralization. The results may be related to prevention of osteoporosis (Kim et al. 2003; Yamaguchi and Uchiyama 2003; 2004; Yamaguchi et al. 2005).

Carotenoids are very sensitive to heat, oxidation, and light, due to their unsaturated chemical structures (Fig. 8.1). They are almost insoluble in water and only slightly oil soluble at room temperature (about 0.2 g/L_{oil}), but their solubility in oil

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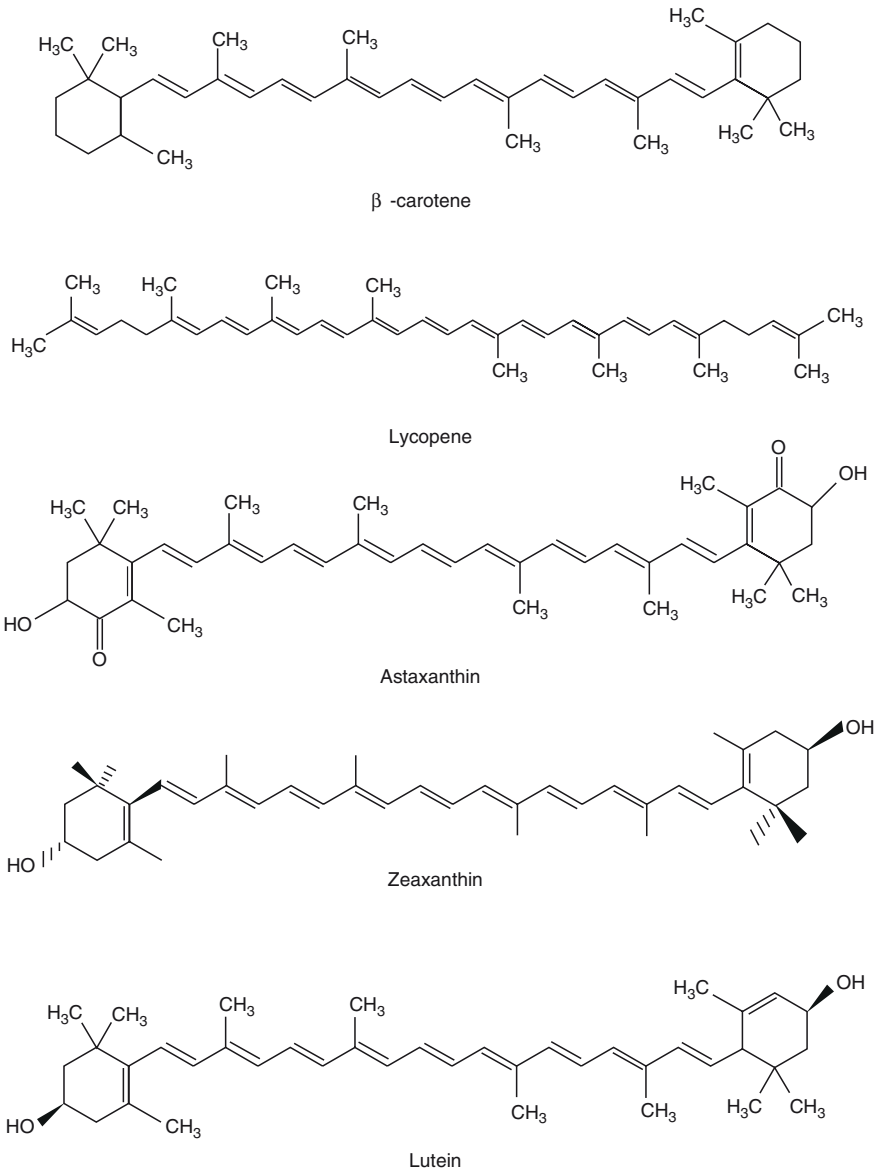

Fig. 8.1 Chemical structures of some carotenoids

increases greatly with increasing temperature (Ax et al. 2001). It has been found that only a minor part of the carotenoids in raw fruits or vegetables is absorbed in the intestines, probably due to the fact that carotenoids in the nature exist as crystals or are bound in protein complexes. In contrast, carotenoids dissolved in vegetable oils show a higher bioavailability (Parker 1997).

Incorporation of carotenoids into micro and nano structures may influence their solubility and crystallinity. After formulating carotenoids into such particulate systems,

they may be easily delivered into cellular compartments, improving their bioavailability. The carotenoids considered in this chapter are β-carotene, lycopene, astaxanthin, lutein, and zeaxanthin (Fig. 8.1) which have been applied for food coloration, and human nutrition due to their antioxidant biological property.

β-carotene is the most widespread of all carotenoids. It is responsible for the orange–yellow color of carrot, palm fruit, pumpkin, mango, acerola, yellow cassava, etc. β-carotene has provitamin A activity. It is an apolar, bicyclic carotenoid that contains 11 conjugated double bonds. The chemical structure of β-carotene is accountable for its high antioxidative activity. β-carotene supplementation may reduce risks of heart disease deaths and stimulate proliferation and differentiation of osteoblast cells (Buijsse et al. 2008; Sahni et al. 2009). Köpcke and Krutmann (2008) have recently reported that dietary supplementation of humans with β-carotene may protect against sunburn.

Lycopene also belongs to the group of carotenoids. It shows a very high quenching rate of reactive singlet oxygen, due to its apolar and acyclic molecular structure. Lycopene is a natural red pigment mainly found in tomato, tomato products, and lycopene-carrots. Epidemiological evidence has suggested that lycopene can protect individuals from colorectal cancer (Vrieling et al. 2007), and men from prostate cancer (Schwarz et al. 2008).

Astaxanthin exist in salmon, lobster, fish eggs, crabs, trout, fish eggs, and flamingo. Its molecule has two cyclic end groups with polar groups, similar to lutein and zeaxanthin.

Lutein and zeaxanthin are isomers found in various foods including green leafy vegetables, fruits, and marigold flower. They show identical chemical formulas with different position of a double bond in one of the end rings. They are present in the human retina. Christen et al. (2008) have shown to possibly reduce the risk of developing cataracts by a diet rich in these carotenoids. Zeaxanthin might be effective to prevent age-related macular degeneration (AMD) (Sajilata et al. 2008).

With modern methods of encapsulation technology, solubility, stability, and bioavailability of carotenoids can be considerably improved (Hoppe et al. 1986; Horn and Rieger 2001; Ax 2003; Ribeiro and Schubert 2004; Engel et al. 2005; Ribeiro et al. 2005a, 2006a, 2008; Schuchmann et al. 2005; Auweter et al. 2006; Flanagan and Singh 2006; End et al. 2007; Feldthusen et al. 2007; Garti and Aserin 2007; Garti et al. 2007; Leuenberger 2007; McClements et al. 2007). In order to achieve optimized physical and chemical stability, and bioavailability of carotenoids in these advantageous formulations, innovative processes for their production have been developed and investigated, which will be discussed in this chapter.

8.2 Processes to Encapsulate Carotenoids

Since carotenoids are very expensive, some cost-effective processes are desirable to produce carotenoid formulations, in order to reduce the capital cost of the final products. The challenge for manufactures is to formulate easy-to-disperse powders, colloidal dispersions, emulsions, or suspensions, which are suitable for various applications.

Optimization of processing conditions may prevent carotenoid oxidation and isomerization, and improve its solubility to achieve satisfactory bioavailability. Choice of optimal process, emulsifier(s), and other ingredients are the most important variables to achieve droplets size and desired color for each application. As differences in particle size may also give rise to variations in color shade, a good process control is essential during the manufacture of coloring-formulations (Müller and Tamm 1966; Runge et al. 1998; Lüddecke et al. 2004a).

8.2.1 O/W Emulsions

Oil-in-water (O/W) emulsions containing carotenoids dissolved in finely dispersed oil droplets (Fig. 8.2) can be produced using various emulsification processes. This type of formulations may increase absorption of these substances in vitro and in vivo. For preparing carotenoid-loaded O/W emulsions, the carotenoid is dissolved in a vegetable oil or in an apolar solvent at elevated temperatures, and subsequently emulsified with an aqueous phase containing an emulsifier to stabilize the droplets. Carotenoids in the forms of fine droplets show a better water dispersibility compared to those in bulk form.

Submicron-sized oil droplets allow for a supersaturation of carotenoid molecules in O/W emulsions (Schweikert and Kolter 1997; Ax 2003). There are two reasons for this as follows:

(1) There is a lack in crystallization germs or seed crystals after emulsification (Bunnell et al. 1958)
(2) The concentration of saturation is significantly increased within small droplets (Mersmann et al. 2005) as described by (8.1):

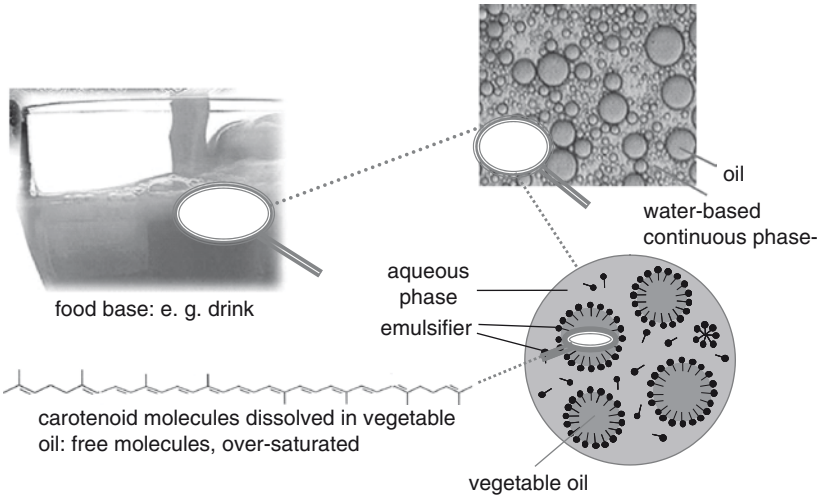

Fig. 8.2 Microstructure of carotenoid-loaded O/W emulsion-based products

$$\ln\left(\frac{c}{c*}\right)=\frac{4\gamma_{CL}M}{RT\rho_c L^*_{crit}}. \tag{8.1}$$

Herein c is the saturation concentration as a function of droplet radius r, c^* the saturation concentration for droplets of infinite radius ($r\rightarrow\infty$ or planar flat interfaces), γ_{CL} is the interfacial tension between the crystals and the liquid phase, R the Avogadro constant, M the molar mass, T the temperature, ρ_c the crystal density, and L_{crit}* is the critical germ forming diameter.

Submicron-sized, vegetable oil-based emulsion droplets are stable against sedimentation and coalescence over long storage time and thus account for stable, homogeneous, and water-dispersable products. In their production, however, some hurdles have to be taken. Fine carotenoid O/W emulsions have been prepared by various methods, such as high-pressure homogenizers, as well as membrane and microchannel emulsification processes, and phase inversion temperature (PIT) method. These will be described here.

8.2.1.1 High-Pressure Homogenization

Challenges in the production of high-pressure homogenized submicron-sized carotenoid-loaded emulsions involve the adaptation of temperature profiles and residence times in each operational unit to the kinetics of carotenoid molecule solution, their degradation and crystallization as well as to the kinetics of droplet breakup, stabilization by interfacial active material and coalescence.

The process line as developed by Ax (2003) and patented in 2005 (Feldthusen et al. 2005) involves the unit operations of (1) carotenoid molecule solution, (2) emulsion premix preparation, (3) high-pressure-homogenization, and (4) emulsion droplet stabilization (Fig. 8.3).

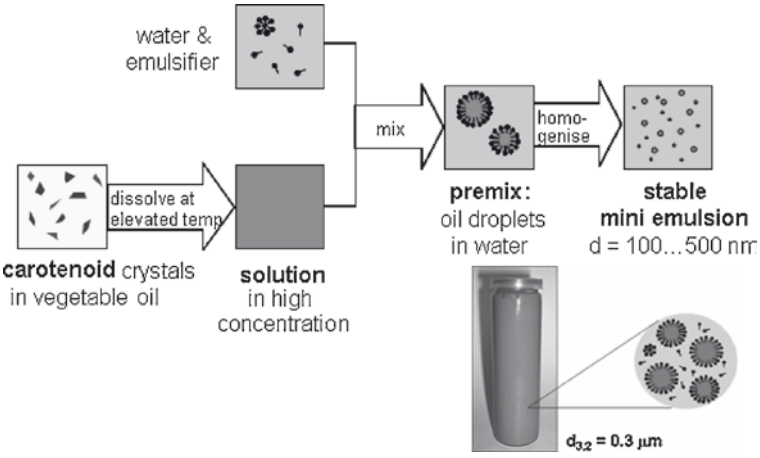

Fig. 8.3 Principle of carotenoid-loaded O/W emulsion preparation with unit operations involved (Ax, 2003) (Courtesy of Shaker Verlag)

For the dissolution of carotenoid molecules in vegetable or middle-chained triglycerides (MCT) temperatures above 100°C are required (Fig. 8.4). At these temperatures dissolution time is limited to some seconds. High temperatures result in structural changes, as depicted by Fig. 8.5. With increasing dissolution temperature a higher amount of diverse *cis*-isomers are found after dissolving *all-trans* isomers of carotenoid molecules in oil and emulsifying them. Therefore, temperatures should be decreased as fast as possible. Most easily this is realized by quenching the carotenoid loaded oil with a cold emulsifier solution, being the continuous phase of the emulsions. In these emulsion premix droplets the carotenoid molecules are oversaturated as their radius is too high for stabilizing them in the dissolved state [see (8.1)]. Droplet sizes have to be reduced without retention time.

High-pressure homogenization is the ideal mechanical process for producing droplet sizes below 1 μm. High-pressure homogenizers have been traditionally used in the dairy industry. These machines are operated continuously at throughputs up to several thousands L/h. They consist essentially of a high-pressure pump and a homogenizing nozzle. The pump creates the pressure, which is then transferred within the nozzle to kinetic energy being responsible for droplet disintegration. Droplet elongation, deformation, and break-up in high-pressure homogenization nozzles are mainly due to elongational and shear stresses as well as inertia turbulent and cavitational stresses acting at the interfaces. Recent developments in high-pressure homogenizing concentrate on nozzle design (Schuchmann 2005, 2007; Schubert 2005). Examples of new homogenizing nozzles (Fig. 8.6) are opposing jets, for example, Microfluidizer® (Cook and Lagace 1985, 1990), jet dispersers (Hovestad et al. 2000), and a simple orifice valve (Stang 1998) as well as developments

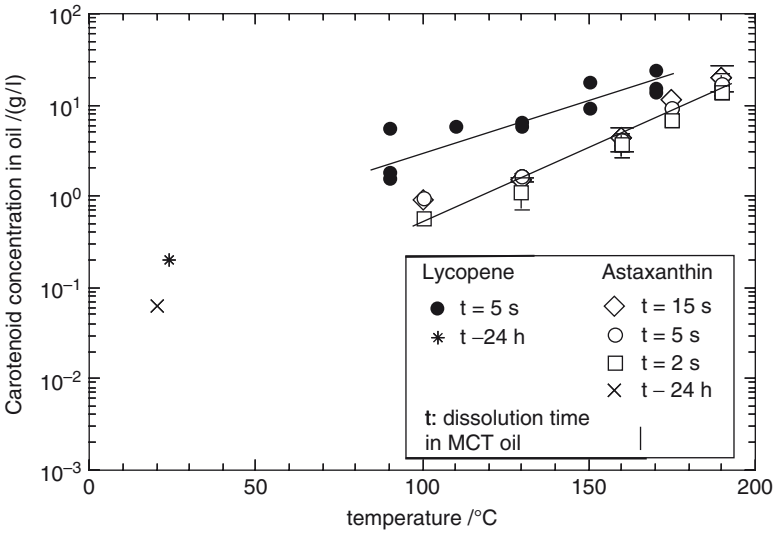

Fig. 8.4 Dissolution of carotenoid molecules in MCT oil: carotenoid concentration as a function of dissolution temperature and time *t* (Ax 2003) (Courtesy of Shaker Verlag)

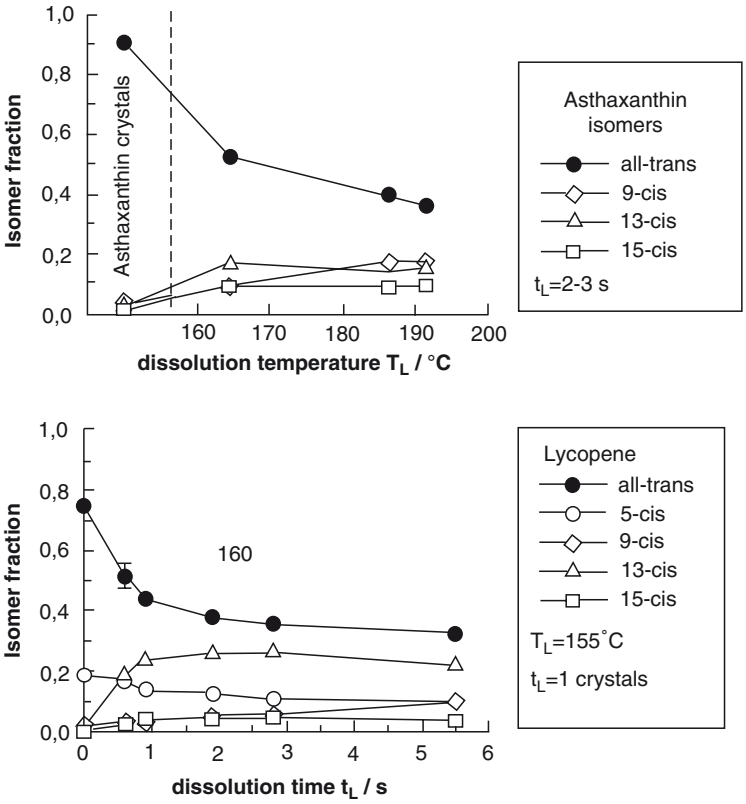

Fig. 8.5 Isomer fractions of astaxanthin and lycopene after dissolution at different temperatures T_L and time t in oil and emulsification (Ax 2003) (Courtesy of Shaker Verlag)

based on it (Kolb 2001; Freudig 2004; Aguilar et al. 2004, 2008; Scheid and Buchholz 2005; Köhler et al. 2007, 2008).

The design of the homogenizing nozzle influences the flow pattern of the emulsion in the nozzle and hence droplet disruption (Stang et al. 2001). In Fig. 8.7, the elongational rate and the kinetic turbulent energy are given for a relatively simple but efficient homogenization nozzle consisting mainly of a simple round valve (Köhler et al. 2008).

Droplet elongation, deformation, and break-up are realized within milliseconds. A huge new interfacial area is created which has to be covered and stabilized by newly adsorbing emulsifier molecules. The adsorption kinetics has to follow break-up kinetics as droplet collision rates are quite high in emulsions of reasonable disperse phase contents (as, for example above 30 vol% in carotenoid emulsions). Therefore at least one emulsifier of small molecular mass and easy molecular structure has therefore to be added, for example, Tween® 20. This emulsifier is responsible for droplet stabilization against re-coalescence within the homogenization nozzle. For O/W emulsions containing carotenoids additional emulsifiers are usually added being required for long-term shelf-life stability and bioavailability (see Sect. 8.3).

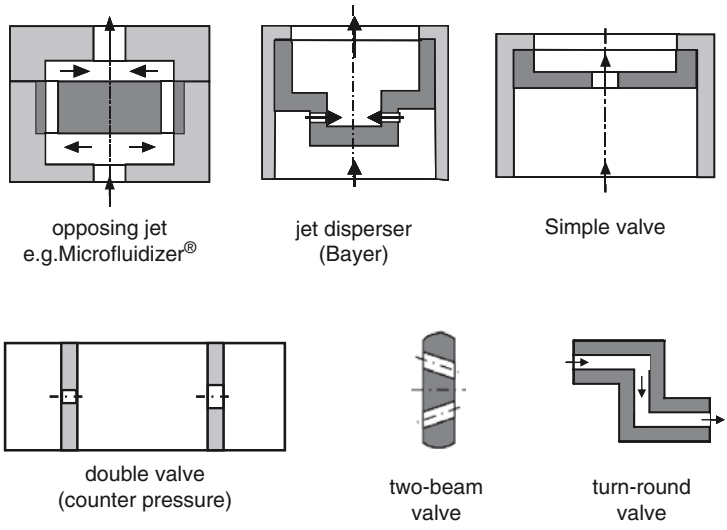

Fig. 8.6 Recent developments in high-pressure homogenizing: New homogenizing nozzles for efficient droplet disruption (Schuchmann 2007)

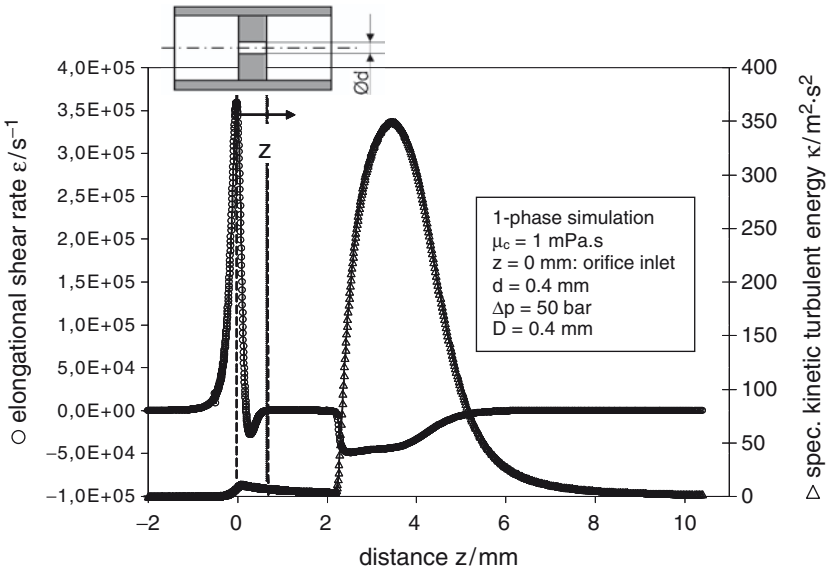

Fig. 8.7 Elongational shear rate and specific kinetic turbulent energy in a simple orifice valve, as required for efficient droplet break-up to the submicron-sized range in homogenization of carotenoid-loaded emulsions (CFD simulation: Fluent®) (Aguilar et al. 2008; Köhler et al. 2007)

Droplet size distribution depend on oil type (viscosity), emulsifiers applied (dynamic interfacial tension), homogenizing nozzle geometry, and homogenization pressure. An example is given in Fig. 8.8.

The carotenoid molecules themselves do not show any relevant interfacial activity and do not change droplet viscosity to a relevant amount. Thus droplet disruption is not influenced by dissolved carotenoid molecules (Fig. 8.9). Absorption spectra depict that carotenoid molecules are mainly found in their molecular dissolution status within droplet of submicron size range (Fig. 8.10) (Ax 2003; Velikov and Pelan 2008).

Solid lipid nanodispersions can also be produced by high-pressure homogenization by melting the lipid at least 10°C above its melting point. Since these kind of particles show a lipid core in solid state, they may provide controlled release of encapsulated lipophilic molecules (Farhang 2007). Hentschel et al. (2008) have successfully applied high-pressure melt-emulsification to encapsulated β-carotene into solid lipid nanodispersions, which particle sizes were smaller than 1 µm.

8.2.1.2 Dead-End Membrane Emulsification

The membrane emulsification process offers the opportunity to produce emulsions with narrow droplet size distribution without high mechanical stress at low energy input (10^4–10^6 J/m^3) compared to conventional mechanical methods (10^6–10^8 J/m^3) (Schubert and Engel 2004). Membranes usually applied in separation processes have been successfully used for emulsification as well. However, this new field of application has just been recently explored (Vladisavljević and Williams 2005, 2006).

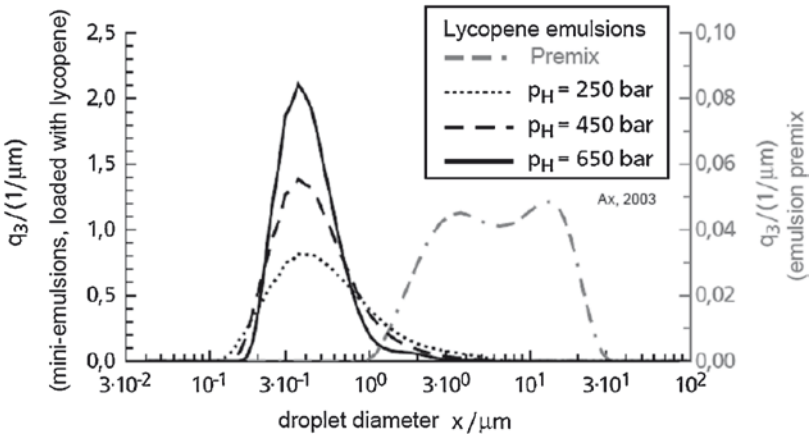

Fig. 8.8 Droplet size distributions (volume density distribution) of lycopene emulsions (premix and high-pressure homogenized at different pressure p_H) (Ax, 2003) (Courtesy of Shaker Verlag)

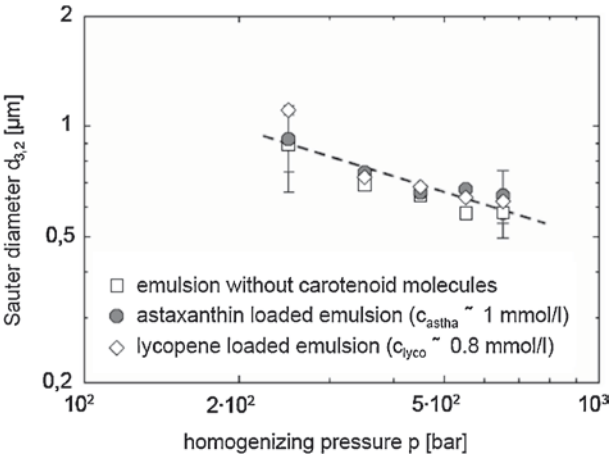

Fig. 8.9 Mean droplet diameter (sauter diameter) of O/W emulsions without carotenoid molecules or loaded with astaxanthin or lycopene, respectively. Varied parameter: Homogenization pressure (Ax 2003)

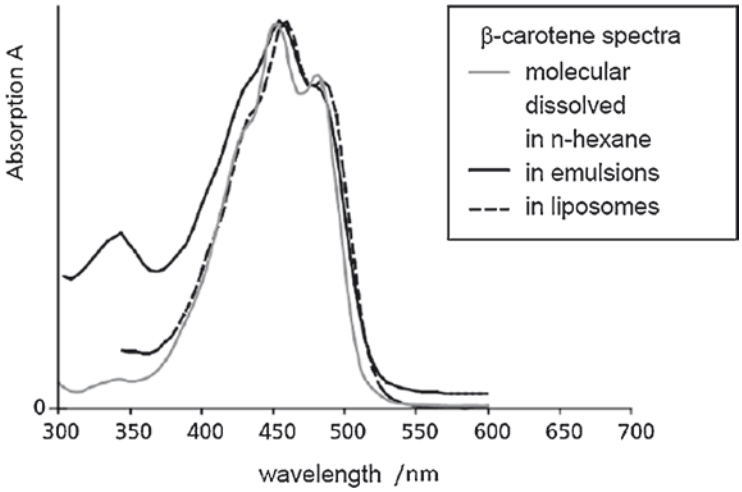

Fig. 8.10 Absorption spectra of carotenoid molecules different formulations: dissolved in *n*-hexane, formulated in submicron-sized O/W emulsion droplets at a concentration well above saturation concentration compared to pure crystals and formulated in liposomes (Ax 2003) (Courtesy of Shaker Verlag)

Premix membrane emulsification is a relative new emulsification process that was developed by Suzuki et al. (1998). In this process, emulsions are produced by pressing a pre-emulsion through a membrane. Defined characteristics of different membranes render this process very flexible. Depending on their pore size and the membranes being hydrophobic or hydrophilic, water-in-oil (W/O) or oil-in-water

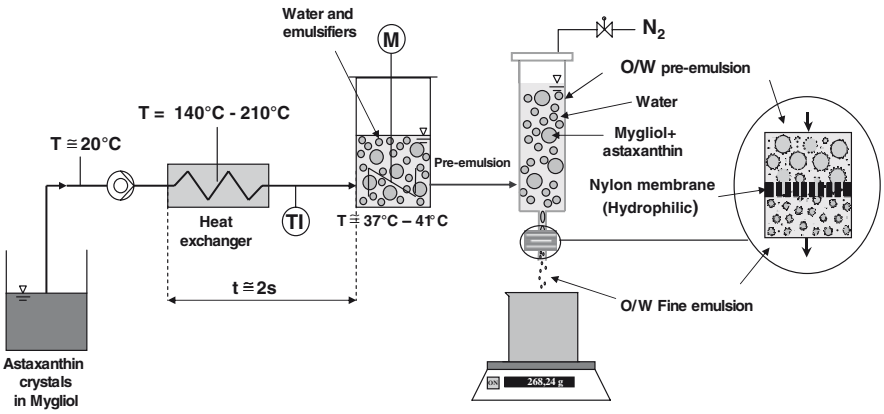

Fig. 8.11 Schematic process for producing O/W emulsions containing carotenoids by premix membrane emulsification (Courtesy of Wiley-Blackwell Publishers)

(O/W) emulsions with different droplet sizes can be produced. The characteristics of membrane emulsification, like low energy input and narrow droplet size distribution, add to other advantages of the premix emulsification compared to direct membrane emulsification, like the facility to clean and disinfect, and high flux. This makes the premix membrane emulsification process very promising and well suited for life science applications.

Ribeiro et al. (2005b) have investigated the effect of repeated premix membrane processing on the microstructure of astaxanthin-loaded O/W emulsions at pressures between 5 and 15 bar and dispersed phase fractions between 10% and 40% (Fig. 8.11). For studying the effect of repeated premix membrane emulsification on emulsion microstructure, the premixes were emulsified three times using the same membrane made of polyamide 6,6. Increased pressure and number of repetitions of the emulsification process resulted in narrower droplet size distributions (Fig. 8.12) and smaller Sauter mean diameters ($d_{3,2}$). Astaxanthin degradation and particle-size distributions were investigated after certain storage times. No coalescence was observed.

8.2.1.3 Microchannel Emulsification

Several studies on microfluidic channels used as an emulsification process have been carried out in the last 10 years. Microchannel (MC) emulsification is a relatively new technique to produce emulsions with a very narrow droplet size distribution (Kawakatsu et al. 1997; Kikuchi et al. 2000; Sugiura et al. 2001; Kobayashi and Nakajima 2006). The fact that narrow droplet size distributions is achieved, without applying strong mechanical forces, makes MC and membrane emulsification interesting methods for shear sensitive components. MC emulsification can be used for producing monodispersed emulsions with a coefficient of variation of less than 5%.

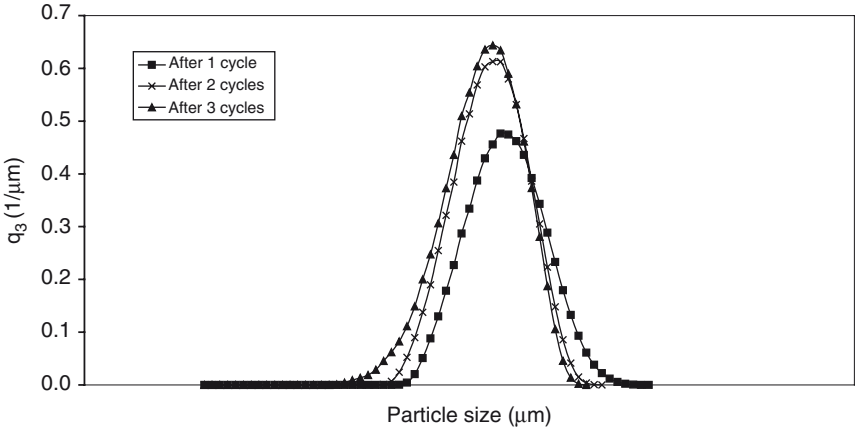

Fig. 8.12 Influence of the repeated premix membrane emulsification on droplet size distributions ($\phi = 0.4$; $\Delta P = 10$ bar)

In this way, microfluidic channel is the most appropriate emulsification process to control the droplet sizes and droplet size distribution of emulsions.

Monodisperse O/W emulsions containing β-carotene has been produced by MC emulsification (Ribeiro et al. 2006a; Neves et al. 2008a; 2008b). The scheme of the process for producing O/W emulsions is shown in Fig. 8.13. The microfluidic channels setup consists of a uniformly sized MC arrays manufactured on silicone microchip, a module, accessory for supplying the continuous and disperse phases, and a microscope video system, which enables the observation of emulsification behavior. A grooved, and an asymmetric through-holes with a slit and circular channels of surface-oxidized, silicon-based microfabricated devices were used for preparing the emulsions. Because of oxidation treatment, MC plates possess negative charge on their surfaces. The grooved type consists of an array of microfluidic channels and a slit-like terrace, which produces droplets in a relatively low throughput between 0.01 and 0.1 mL/h. The asymmetric straight-through microchannel plate used consists of channels with a diameter of 10 μm and slits with a length of 50 μm in longer line and 10 μm in shorter line. The flow rate of the continuous and disperse phases were 20 mL/h and 0.5 mL/h, respectively. Laminar flow rates of disperse and continuous phases are very important, independent variables for controlling the droplet size (Vladisavljević et al. 2006). Sucrose laurate was used as the emulsifier.

Figure 8.14 shows images of β-carotene droplets generation during and after MC emulsification using grooved (a) and straight-through (b) types, respectively. As can be seen, MC emulsification with well-defined channels provides formation of monosized O/W emulsions containing lipophilic compounds, such as β-carotene. Monodispersed droplets with an average diameter of approximately 9 μm and 27 μm, and coefficient of variation of about 5.0% and 2.6%, could be produced

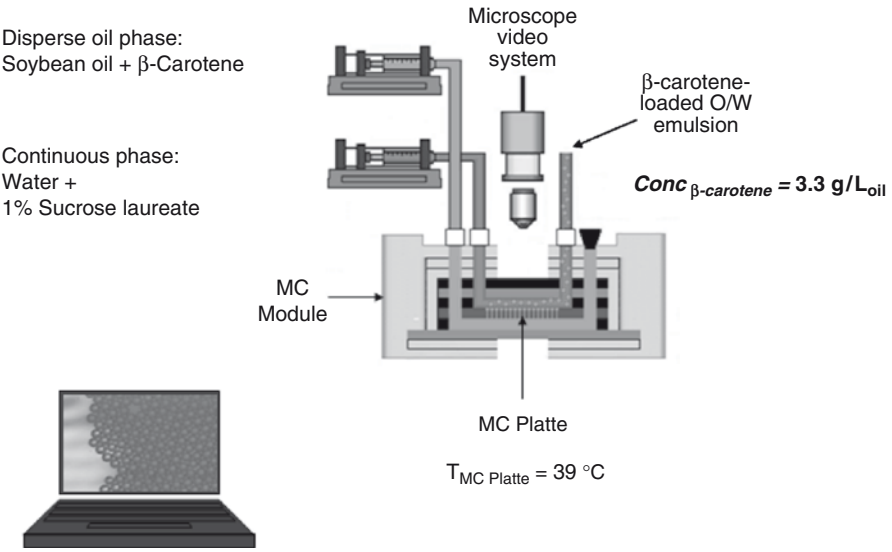

Fig. 8.13 Scheme of the microfluidic channels for monosized droplet formation

using grooved and asymmetric straight-through MC plates, respectively. Due to the hydrophilic surface of the silicon-oxidized microchannel plate, β-carotene-loaded O/W emulsions could be successfully prepared. Total β-carotene concentration in the oil phase was 3.3 g/L_{oil}. Because of added α-tocopherol, β-carotene concentration remained unchanged for more than 3 weeks. Emulsions were chemically and physically stable for at least 5 months at 4°C.

8.2.1.4 Phase Inversion Temperature Method

Oil-in-water (O/W) emulsions containing ethoxylated non-ionic emulsifiers may undergo a phase inversion to water-in-oil (W/O) emulsions upon heating. At the phase inversion the hydrophilic and lipophilic properties of the emulsifier are balanced, resulting in minimum interfacial tensions between oil and water phases. This temperature-induced phase inversion is utilized in the so-called PIT method. This technique is a thermodynamic phenomenon that produces nanodispersed bluish transparent O/W emulsions with narrow droplet size distributions (Engels et al. 1995).

Non-food-grade, nanosized carotenoid-loaded O/W emulsions were successfully produced using cetostearyl poly(oxyethylene (12) glycol) and glyceryl monostearate as the emulsifying agents by PIT method (Ribeiro and Schubert 2004). Dycapryl ether was used as the oil phase. The PIT emulsion was prepared conventionally by heating up to 74°C and subsequently rapid cooling down to 25°C. The total astaxanthin and lycopene concentration was around 0.3 and 3.5 g/$L_{emulsion}$. No detectable degradation of lycopene and astaxanthin was observed

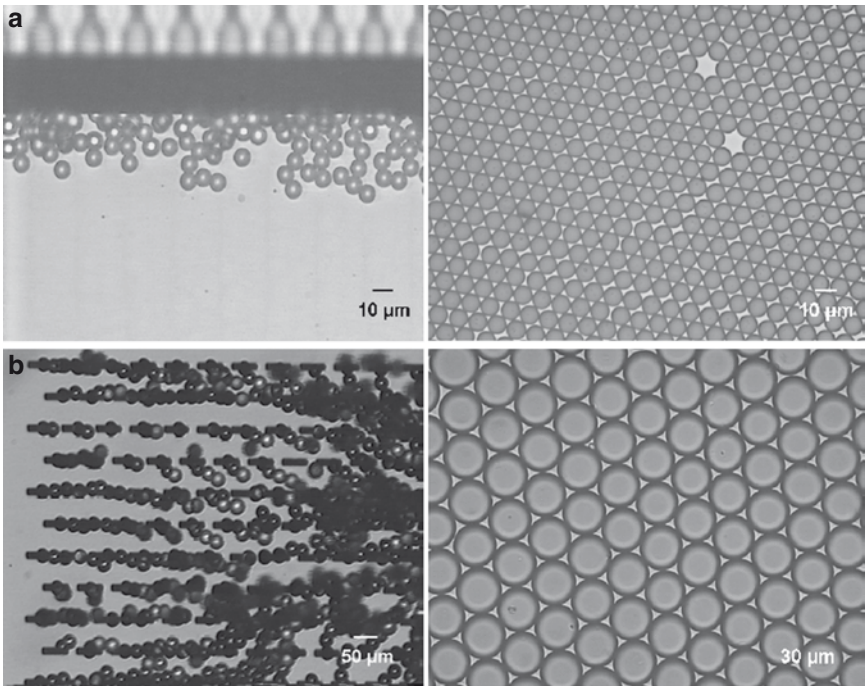

Fig. 8.14 Images of the droplets formation during and after emulsification process using two different types of MC plates: (**a**) grooved and (**b**) asymmetric straight-through

during a month of storage. The Sauter mean diameter of the finely dispersed droplets was around 80 nm and droplet size distributions were between 40 and 200 nm, as determined by means of laser diffraction combined with polarization intensity differential scattering (PIDS) technology (Fig. 8.15). These data suggest that PIT emulsions could be an excellent vehicle for delivering carotenoids.

8.2.1.5 High Internal Phase Emulsions (HIPE)

The continuous phase of an O/W emulsion can be extracted through centrifugation or evaporation under vacuum. This yields a transparent, gel-like cream which can be stored and later redispersed in water. The oil droplet deforms into polyhedral structures, separated by extremely thin films of emulsifier, which can be redispersed in water. Thus, the cream is called highly concentrate emulsion or "biliquid foam".

Ribeiro et al. (2005a) have produced biliquid foams containing carotenoid in submicron size. Fine emulsions were prepared by mixing vegetable oil with aqueous solutions of Tween® 20 and Xanthan by high-pressure homogenization (Fig. 8.16). The average droplet diameter was controlled by changing the

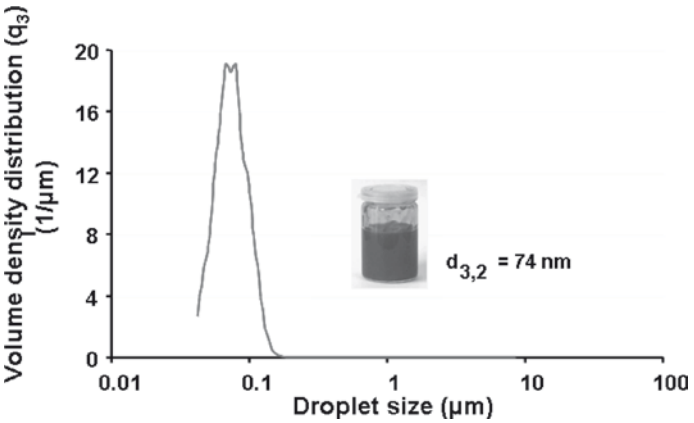

Fig. 8.15 Droplet size distribution of lycopene O/W emulsion and sample appearance

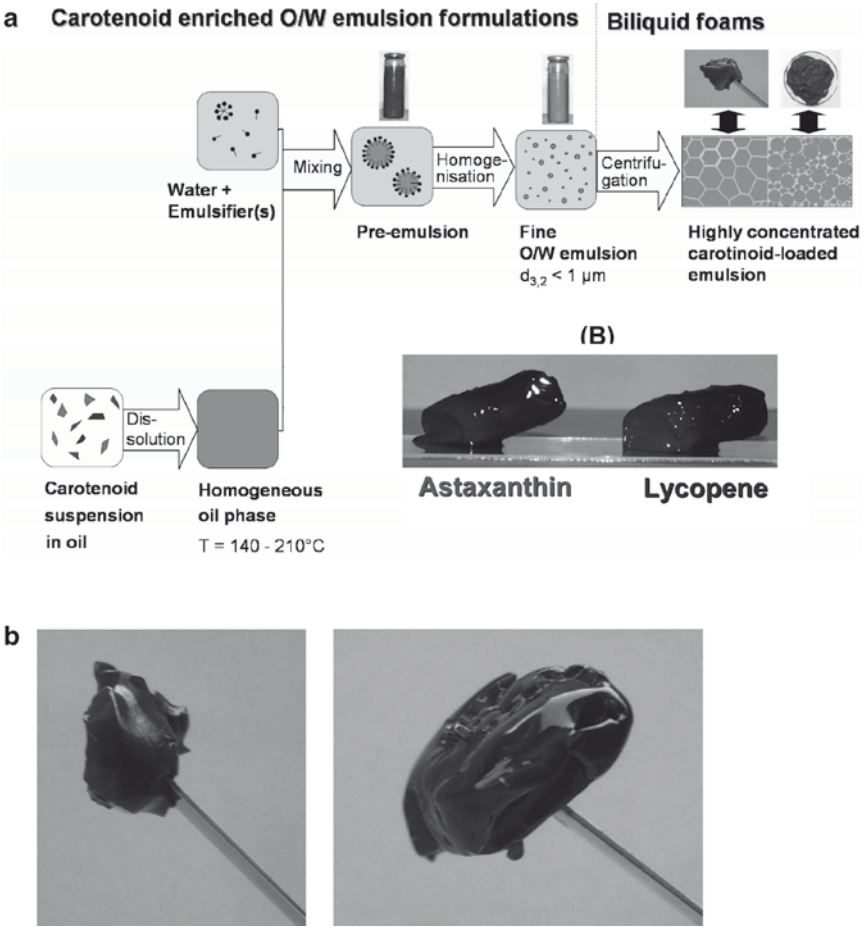

Fig. 8.16 (**a**) Schematic procedure for producing biliquid foams, (**b**) appearance of the samples

emulsifier-to-oil ratio. It could be set in the range from 100 to 800 nm by choosing the concentration of the disperse phase between 10% and 50%. The aqueous phase of the emulsions was removed by centrifugation. After centrifugation, the cream turns into a biliquid foam with an oil volume fraction up to 0.99, which shows a transparent and elastic (gel-like) structure. After centrifugation the droplet still retained their integrity and did not coalesce. The most important factors that have been identified are the physical properties of the continuous and dispersed phase as viscosity and the droplet size distribution before and after centrifugation.

8.2.2 Liposomes

As described in Chap. 2, liposomes consist of a molecular double layer that separates an inner, watery phase from the outer, continuous watery phase. Except from the inner part of the spherical particle not being "empty" but filled with a watery phase, the structure of liposomes could best be described as hollow spheres. With both the inner and outer phase of liposomes being hydrophilic, the system seems to be suited for encapsulation of water soluble active agents.

A closer look into the structure of the shell of these hollow spheres, the molecular double layer, reveals the opportunity for the formulation of lipophilic and amphiphilic active agents in a dissolved state in this system (Senior 1987). For applications in food systems, the molecular double layer of liposomes is made of phospholipid molecules, commonly known as lecithins, of high purity. Upon hydration and input of mechanical energy, these molecules arrange themselves in double layers with their lipophilic parts oriented towards the center and their hydrophilic parts making up the outer surface of the double layer. Since, at the edge of these double layers, the inner, lipophilic part gets into contact with the watery phase, the free energy can be reduced by the formation of hollow spheres, that is, liposomes. With a lipophilic active agent present during the formation of the double layer and the liposomes, it can be incorporated into the double layer's lipophilic core. There, it is dissolved in the lipophilic parts of the phospholipid molecules.

A formulation of lipophilic active agents in liposomes can be advantageous for several reasons. First of all, the active agent is formulated as a solution, thus exhibiting a high bioavailability. Furthermore, since liposomes form spontaneously upon hydration of the phospholipid, they have a good physical stability over time. Depending on the process chosen for production, their size can be adjusted even to diameters below 100 nm so that the water-dispersible formulations have an almost clear appearance (Fig. 8.17). Several processes for production of liposomes are described in literature (Lasic 1993; Winterhalter and Lasic 1993).

For the formulation of lipophilic active agents in liposomes in general, the active agent has to be dissolved in the substance that will later, upon hydration, build the double layer. The solubility of carotenoids in any phospholipid fraction at ambient conditions is by far too low in order to achieve reasonable carotenoid

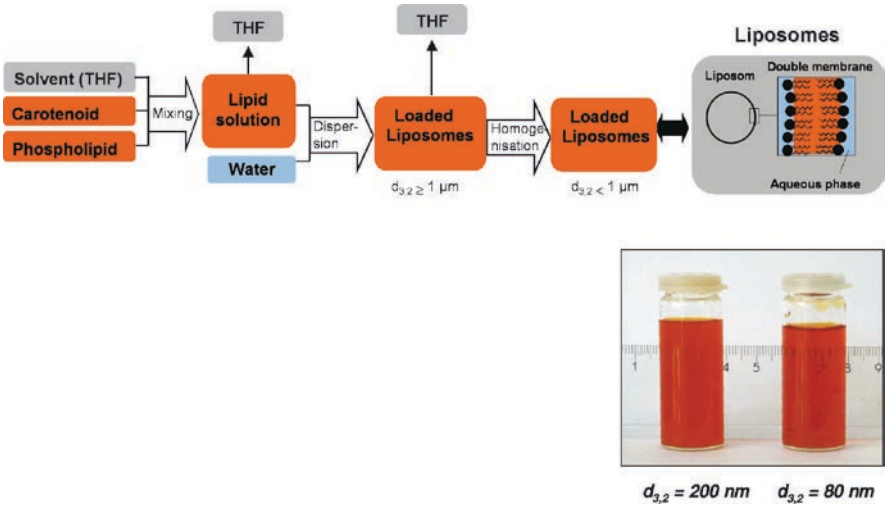

Fig. 8.17 Carotenoid-loaded liposomes formulation and appearance of liposomes containing lycopene (Ax 2003) (Courtesy of Shaker Verlag)

concentrations in the final product. Although solubility can usually be increased at elevated temperatures, this method is unsuitable for reaching sufficient carotenoid concentrations. Phospholipids decompose at approximately 180°C before a satisfactory concentration of carotenoids can be achieved. Therefore, a solvent which is capable of solving reasonable concentrations of both the phospholipid and the active agent, that is, the carotenoid, must be found. Above that, as will be described in a later step, the solvent's vapor pressure in the temperature range up to approximately 100°C should be high enough for allowing its removal from the solution under reduced pressure.

The processes for production of liposomes known from literature could be adapted for the production of carotenoid-loaded liposomes as described by Ax (2003) and in the following. Having selected an appropriate solvent, a solution of phospholipids, the active agent, and, if necessary, an antioxidant, is prepared. From this solution, the solvent is extracted, for example, in a rotary evaporator, until a semi-solid concentrated solution is obtained, which is usually the case at a solvent concentration of approximately 60%. By means of introducing mechanical energy and high shear, this solution is dispersed in water. In this step, liposomes form spontaneously. In order to obtain the desired liposome size distribution, this dispersion is subjected to high-pressure homogenization, for example, in a microfluidizer. Any excess solvent is then extracted from the formulation by nitrogen-stripping. The resulting liposome dispersion has a clear appearance if the size of the liposomes is below 100 nm, which can be achieved by applying a homogenization pressure of 1,000 bar or higher, depending on the homogenization valve (Ax et al. 2000).

Solvents identified as suitable for producing carotenoid-loaded liposomes are chloroform or tetrahydrofuran (THF). Carotenoid concentrations in the final

liposome-dispersion are reported to have reached 0.3 mmol/L at a phospholipid concentration of 10%. The remaining solvent concentration was below 0.05%. By comparison of the absorption spectra of carotenoids dissolved in hexane and formulated in liposomes, it could be proven that the carotenoids existed in a dissolved state in the double layer of liposomes (see Fig. 8.10; Ax 2003).

The maximum achievable concentration of active agents in liposomes greatly depends on their polarity that determines their position in the double layer. Less polar carotenoids like β-carotene and lycopene tend to accumulate in the core of the double layer, whereas more polar structures will orient their polar groups away from the double layer's center. Therefore, higher concentrations of polar carotenoids than of less polar ones can be incorporated into liposomes (Grolier et al. 1992; Socaciu et al. 2000; Barenholz et al. 2006).

The need for organic solvents with rather well-specified properties renders production of liposomes for food applications difficult if not impossible. Above that, the highly purified lecithin essential for the formation of double layers and liposomes is very expensive compared to most food ingredients used in concentrations around 10% in formulations for active agents in food systems. Until now, only cell culture studies have been carried out in order to measure the bioavailability of carotenoids from liposomes. In these trials, the bioavailability from liposomes was superior to most other formulations like O/W emulsions. Due to the high specific surface of the disperse liposome-system, this is not surprising. However, levels of bioavailability of carotenoids in O/W emulsions stabilized with certain emulsifier systems by far exceeded those reported for carotenoids in liposomes. Bioavailability and stability issues will be further dealt with in Sect. 8.3.

8.2.3 Production of Nanoparticles by Precipitation

Nanoparticles containing carotenoids can be produced by precipitation and condensation processes with remarkable droplet size from 100 to 300 nm (Horn 1989; Paust 1991, 1994; Horn and Lüddecke 1996; Auweter et al. 1999; Köpsel 1999; Horn and Rieger 2001; Lüddecke & Schweikert, 1999; Lüddecke et al. 2004b). There are three different methods applied for producing carotenoid-loaded organic nanoparticles: Emulsification evaporation, emulsification diffusion, and solvent-displacement methods. Emulsification evaporation technique is applied when an apolar organic solvent is used. Solvent-displacement method is appropriated when a polar solvent (acetone, ethanol) is used as the dispersed phase to produce O/W nanoemulsions containing carotenoids. The nanodroplets formation process is related to the decreasing droplet size due to the rapid diffusion of solvent to the aqueous phase. Emulsification diffusion method, which uses amphiphilic solvent (benzyl alcohol, ethyl acetate) as the dispersed phase, could also be successfully applied to produce nanoemulsions containing carotenoids with remarkable narrow droplet size distributions and small droplets size. Solvent-displacement and emulsification diffusion methods are very low energy-input processes.

Ribeiro et al. (2008) have produced non-food grade nanoparticles containing β-carotene by interfacial deposition of the biodegradable polymers, poly(D,L-lactic acid) (PLA) and poly(D,L-lactic-co-glycolic acid) (PLGA), following displacement of acetone from the organic phase towards the aqueous phase (Fig. 8.18). In this kind of formulation, the polymer assumes the function of protective colloid, providing physico-chemical stabilization of the nanodispersed particles. The total β-carotene concentration in the dispersed phase was about 150 mg/L. The results showed that polymer-encapsulated β-carotene nanodispersions, with approximately 100% entrapment efficiency, could be prepared by the solvent-displacement method. Sauter mean diameters of the nanodroplets were found to hardly vary using gelatin or Tween® 20 as emulsifiers, with a $d_{3,2}$ of about 70 nm and narrow particle size distributions in both cases (Fig. 8.19). After freeze-drying, the particles were resuspended in water, showing no changes in the droplet sizes and their distribution.

Chu et al. (2007) have prepared milk protein-stabilized β-carotene nanodispersions using acetone. Particles stabilized by sodium caseinate have shown a bimodal size distribution from 13 to 171 nm. Morphological experiments confirmed the presence of particles stabilized by casein micelles and submicelles, which 88% of the β-carotene precipitated was stabilized by submicelles. Similar research work has been patented by Auweter et al. (2006), where carotenoids were suspended in an azeotropic isopropanol/water mixture. Temperature and pressure were applied for allowing the complete dissolution of carotenoid crystals. The homogeneous organic solution was mixed with sodium caseinate solution. Nanoparticles around 100 nm were produced, then filtered and freeze-dried.

Bortlik et al. (2005) patented a highly bioavailable whey protein-based formulation which may be applied to encapsulate carotenoids. Polar organic solvent were used for dissolving the active molecule. When lycopene was encapsulated into the

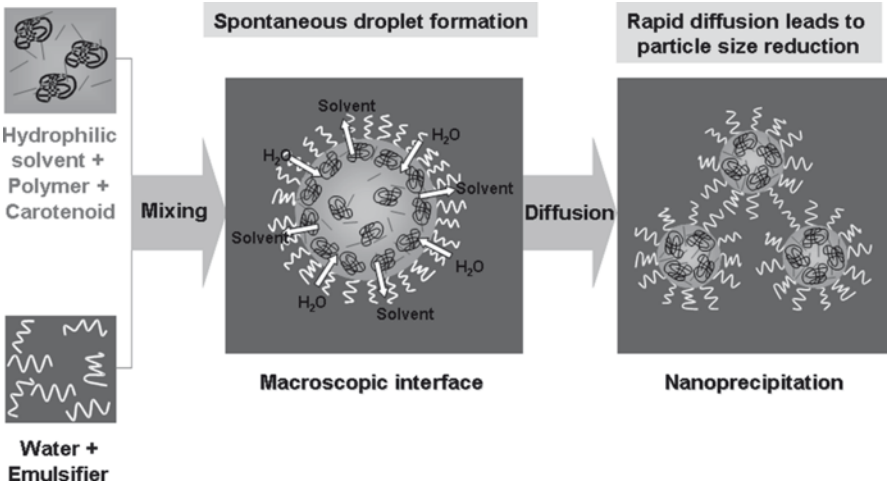

Fig. 8.18 Schematic representation of the spontaneous formation of β-carotene nanoparticles by solvent displacement method

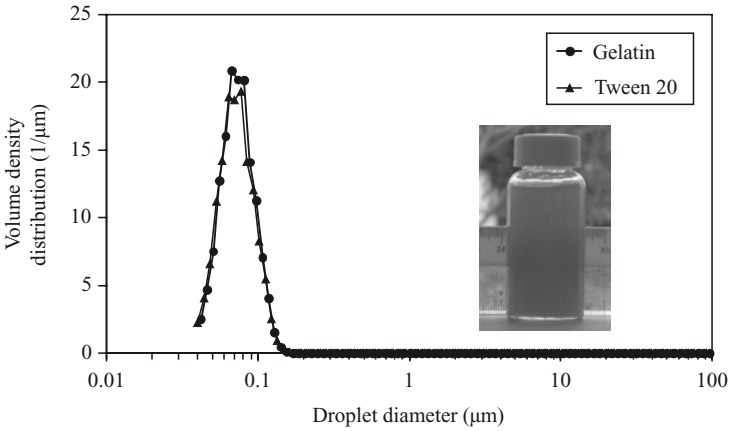

Fig. 8.19 Droplet size distribution of β-carotene nanoparticles stabilized with gelatin or Tween® 20, and appearance of a sample with gelatin (Ribeiro et al. 2008)

whey protein matrix, called "lactolycopene", it exhibited similar bioavailability in plasma and buccal mucosa cells in humans compared to tomato paste (Richelle et al. 2002).

Solvent evaporation technique has been used to microencapsulate astaxathin in a chitosan matrix cross-linked with glutaraldehyde (Higuera-Ciapara et al. 2004). Microcapsules were chemically stable over a storage time of 8 weeks.

These methods show some advantages, such as spontaneous particle formation, consequently low energy input, high entrapment efficiency, and high reproducibility.

8.2.4 Nanoparticles Produced by RESSAS Method

Rapid expansion of supercritical solution into aqueous solution (RESSAS) is a novel process for producing and stabilizing nanoparticles in aqueous solution. Stable suspension of carotenoid nanoparticles can be produced by RESSAS. In this method, a mixture of a poorly water soluble substance and a supercritical fluid, such *a*s trifluormethane (CHF_3), carbon dioxide (CO_2), or nitrous oxide (N_2O), is expanded into an aqueous solution containing a surfactant, which may impede growth and/or agglomeration of nanoparticles which can happen due to collisions in the capillary nozzle into the expansion chamber (Fig. 8.20) (Türk and Lietzow 2004).

Astaxanthin particles below 300 nm have been formed by RESSAS process using supercritical CO_2 ($p_c = 74$ bar, $T_c = 31°C$) (Fig. 8.21), although their particles show a very low concentration in the aqueous solution containing Tween® 20. Similar results have been observed after production of astaxanthin nanoparticles using supercritical CHF_3, and N_2O. According to Horn and Rieger (2001), carotenoids show poor solubility in supercritical fluids, and they may also promote carotenoid oxidation. Sakaki (1992) investigated the solubility of β-carotene in

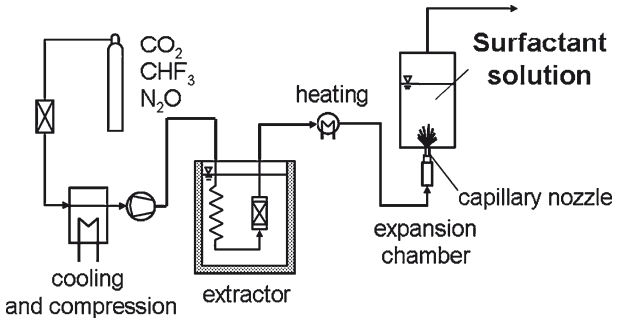

Fig. 8.20 Schematic RESSAS process (diameter of the capillary nozzle = 50 μm)

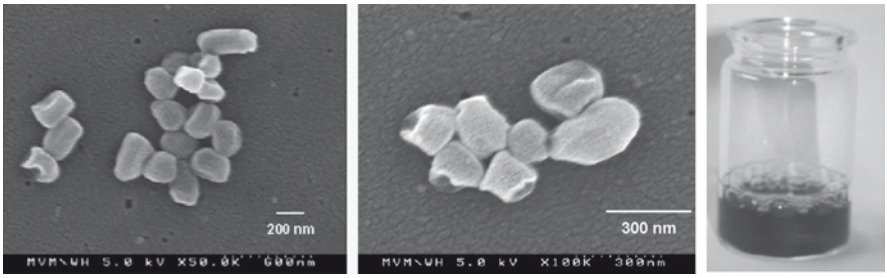

Fig. 8.21 SEM images of astaxanthin nanoparticles and appearance of the sample

supercritical CO_2 and N_2O at different pressure (9.64–29.5 MPa) and temperature (308–323 K) ranges. The maximal solubility of β-carotene in supercritical CO_2 and N_2O was about 5.3 and 66 g/m^3 at 29.5 MPa and 323 K, respectively.

8.2.5 Carotenoid Formulation Using Cyclodextrins

Cyclodextrins are able to serve as host molecules to encapsulate many food ingredients (Shahidi and Han 1993; Hedges et al. 1995), including carotenoids as well (Matioli and Rodriguez-Amaya 2003; Reuscher et al. 2004; Blanch et al. 2007). Complex inclusion of carotenoid with (the non-food grade) hydroxypropyl-β-cyclodextrin can also be successfully produced (Yuan et al. 2008). Cyclodextrins can be used to increase solubility of active molecules and improve their absorption, protect against light and oxygen, and may prevent off-flavor.

Supercritical fluid extraction and a conventional method using an amphiphilic organic solvent were used for entrapping lycopene into cyclodextrin molecules. Better encapsulation yield was observed by the conventional method using solvent (94% vs. 68%). β-Cyclodextrin–lycopene complex was chemically and physically stable

over 6 months (Blanch et al. 2007). Reuscher et al. (2004) coated γ-cyclodextrin–lutein complexes with a vegetable oil or a biopolymer (e.g., hydroxypropyl methylcellulose) to enhance their stability against oxidation. γ-Cyclodextrin complexes contained approximately 5–25% carotenoid. They also claim that the cellular uptake of lutein was much higher from a cyclodextrin complex than when using micelles or liposome formulations.

Anantachoke et al. (2006) have established a method using a spinning disk device to produce controlled size nanoparticles containing β-carotene with maximal mean diameter of 82 nm. Either α- or β-cyclodextrin or sulfonato-calixarenes were used as stabilizer. Nanoparticles stabilized by cyclodextrins can be easily applied in food products.

An innovative carotenoid self-assembled rigid-rod β-barrel has been developed by Baumeister and Matile (2000). Carotenoids were entrapped in a hydrophobic interior of rigid-rod lipocalin, composed by oligo(*p*-phenylene) peptides. The encapsulation stoichiometry of peptides:carotenoid was 4:1 or 1:1 for β-carotene or astaxanthin, respectively.

8.2.6 Encapsulation of Carotenoids in Carbon Nanotubes

A hypothesis supported by a recent study has shown that degradation of β-carotene can be delayed after its encapsulation into the cavity of single-walled carbon nanotubes (SWCNT) (Yanagi et al. 2006). Although SWCNT cannot be applied in foods, the technique may contribute to other types of application of carbon nanotubes as carrier system of active molecules.

8.2.7 Encapsulation of Carotenoids in Microspheres

The entrapment of carotenoids in microspheres has not been frequently reported in the literature, most likely since it might not be the best choice to improve carotenoid stability and bioavailability.

Furcellaran beads have been reported as an efficient matrix to encapsule β-carotene (Laos et al. 2007); however, a low concentration of β-carotene has been achieved in this system. Beads were produced by furcellaran gelation. First, hot furcellaran solution (4–8%) with sea buckthorn juice was dropped into a 1.3 M salt solution containing either $CaCl_2$ or KCl, allowing its complete gelation. Afterwards, the spherical gel beads ($d = 4.6$ mm) were filtered and washed. KCl solution provided firmer gel beads than $CaCl_2$ solution.

Dunaliella salina, a pink micro-algae rich in β-carotene, was encapsulated in calcium alginate beads and rapidly dried by fluidized bed (Leach et al. 1998). The dry process caused isomerization of *all-trans* carotenoids, which is expected due to temperature sensitivity showed by carotenoids in general. Alginate concentration played an important role on carotenoid stability over storage time.

Kittikaiwan et al. (2007) have developed a novel encapsulation method using chitosan (80 mol% deacethylation) to encapsulate *Haematococcus pluvialis*, a rich source of astaxanthin. *H. pluvialis* beads were coated five times with chitosan solution forming multilayers of chitosan film. Chitosan capsules containing the algal have shown uniform shape with diameters from 0.35 to 0.5 cm and 100 μm film thickness. Storage studies on chemical stability have proven that chitosan as wall material can prevent degradation of astaxanthin.

8.2.8 Production of Microcapsules by Drying Processes

Drying can also be considered as an important unit operation to transform moist or fluid carotenoid formulations into dry solids. It allows the production of carotenoid-microcapsules of a certain structure, size, and shape (Horn et al. 1985; 1988; Cathrein et al. 1991; Vilstrup et al. 1997). Colombo and Gerber (1991) have reported several drying processes to encapsulate carotenoids and fat-soluble vitamins, such as drum-cooling and spraying processes. In this section, the use of spray-drying and freeze-drying to obtain dry carotenoid formulations are discussed in more detail.

8.2.8.1 Spray-drying

Rodríguez-Huezo et al. (2004) have successfully produced dried O/W and W/O/W multiple emulsions containing oleoresin from red chilies and marigold petals by spray drying. Gellan and mesquite gum, maltodextrin, and gum arabic were used as wall materials. Morphological studies have shown microcapsules with large central voids when W/O/W emulsions were prepared with high solid contents (35%); they have shown inner and outer particles sizes ($d_{4,3}$) of 1.5 and 34 μm, respectively.

Reuscher et al. (2004) spray-dried complexes of lutein and γ-cyclodextrin, followed by spraying with a hydroxypropyl methylcellulose coating or dispersion in oil. They claimed enhanced stability of the carotenoid against oxidation by these oil or biopolymer coating.

Spray-dried lycopene-loaded O/W emulsion stabilized with a gum arabic and sucrose mixture (8:2) has been studied as photostabilizer of vitamins A and D3 in skimmed milk (Montenegro et al. 2007). Vitamins degradation has slowed down by 45% in presence of lycopene formulation.

Loksuwan (2007) has encapsulated β-carotene into three different wall materials: Acid-modified and native tapioca starch, and maltodextrin. Use of modified tapioca starch provided higher retention of β-carotene (82%) during spray drying process and resulted in a wider particle size distribution (75–150 μm) but smaller particle size than native tapioca starch and maltodextrin. Chen et al. (2004) have patented a spray-dried formulation in which carotenoids and other fat-soluble molecules have been successfully entrapped in a carbohydrate matrix. Another patent

(Leuenberger et al. 2006) describes the process for preparing beadlets containing lipophilic compounds, for example carotenoids. Emulsions stabilized by gelatin were produced and converted to dry powder using spray drying. Afterwards, the gelatin matrix in the coated particles was cross-linked by exposure to radiation. In another process, after preparing emulsions, a cross-linking enzyme was added, then emulsions were spray-dried and the gelatin matrix was cross-linked by incubating.

Lycopene macrocapsules with a combination of gelatin and sucrose as wall materials have been produced by Shu et al. (2006). Emulsions were previously prepared by high-pressure homogenization and then spray-dried. Encapsulation yield and efficiency varied with the ratio of core and wall (M_{core}/M_{wall}), ratio of gelatin and sucrose (M_g/M_s), homogenization pressure, temperatures during drying process, and lycopene purity. The highest values of encapsulation yield and efficiency found were about 91% and 82%, respectively. Lycopene slightly degraded after spray drying.

Santos et al. (2005) have encapsulated paprika oleoresin in either a combination of rice starch/gelatin or arabic gum microcapsules with particle sizes of 20 μm or 16 μm, respectively. Rice starch/gelatin as wall materials resulted into porous microparticles, which provided low effective barrier against carotenoid oxidation.

New combinations of carbohydrates and proteins have also been investigated to encapsulate carotenoids. For example, Feldthusen et al. (2005) firstly dissolved astaxanthin in organic solvent and then mixed it with an aqueous phase containing one of the following combinations: trehalose/sodium caseinate, lactose/sodium caseinate, lactose/soy bean protein, or glucose syrup/sodium caseinate. Afterwards the dispersions were spray-dried and the dried product contained 10–25% astaxanthin. Polysaccharides, such as acacia gum, pectin, cellulose, cellulose derivates, and/or modified polysaccharides, are other examples of wall materials of microcapsules containing carotenoids (Leuenberger et al. 2008). Milling and spray-drying processes were used for the preparation of the encapsulated systems.

8.2.8.2 Freeze-Drying

β-Carotene encapsulated in a trehalose-gelatin matrix with and without magnesium chloride ($MgCl_2$) by freeze-drying was studied by Elizalde et al. (2002). First, a coarse β-carotene-loaded O/W emulsion was prepared using a rotor-stator system, and then it was frozen in liquid nitrogen (−190°C) before freeze-drying. Although $MgCl_2$ was added to delay trehalose crystallization and improve microcapsule stability against enzymes, no positive effect was observed. Amorphous phase played an important role on carotenoid stability, and a noncrystallized sample showed 80% retention of β-carotene for at least 6 months. Crystallization of the sugar matrix was intimately related to the loss of β-carotene.

Chiu et al. (2007) reported an interesting encapsulation study done with 4.8% lycopene extract from tomato pulp waste. 4.5% Gelatin and 10% poly(γ-glutamic acid) (γ-PGA) were applied as carrier matrix. Results showed that microcapsules around 39 μm were obtained, and freeze-drying caused a loss of 23.5% lycopene. As expected, different pHs affected the total lycopene release and isomers profile.

In another example, using a combination of drying processes, O/W emulsion containing β-carotenoid stabilized by low methoxylated pectin was atomized into aqueous solution of calcium salts. The microgels were then centrifuged and freeze-dried to yield the final product containing about 2% of β-carotene (Carle et al. 2004). Desobry et al. (1997) compared three drying processes to encapsulate β-carotene with maltodextrin. Before drying, β-carotene was dispersed in 40% maltodextrin solution and high-pressure homogenized three times at 253 MPa. Spray, drum, and freeze-drying provided particle sizes of approximately 30 μm, 105 μm, and 80 μm, respectively. These processes operate at different residence times and temperatures, consequently distinct dried structures were obtained. The highest β-carotene degradation (14%) was observed with drum-drying. Freeze-drying provided less loss of the carotenoid, about 8%.

8.3 Carotenoid Product Formulations

A wide range of processes to encapsulate carotenoids have been previously discussed in Sect. 8.2. Nutritional enhancement of food products with carotenoids will only be useful if they are able to be taken up by the body.

The bioavailability of carotenoids might depend on the following:

(a) Chemical and physical stability of the carotenoids upon storage within the food product
(b) Stability of the carotenoids during the gastro-intestinal tract passage
(c) Uptake by intestinal cells

The next sections will review these three aspects, using emulsion and liposome formulations as examples.

8.3.1 Chemical and Physical Stability of Carotenoids in Food Formulations

Experiments investigating both physical and chemical stability of carotenoid emulsions have been carried out. Droplet size and its distribution is an important characteristic of carotenoid dispersions, which is associated with many properties, such as shelf-life stability, homogeneity, creaminess, viscosity, carotenoid bioavailability, and color. Small oil droplets with supersaturated carotenoids may be kept stable over long storage time (Figs. 8.22 and 8.23), as well as liposomes (Matsushita et al. 2000).

Food products containing carotenoids and providing health benefits by improving antioxidant levels in individuals, so-called “functional foods”, are, for example, beverages, breads, candies, cookies, dairy products (ice-cream, cheddar cheese, etc.), dry cake mix, salad dressings, macaroni products, mayonnaise, savory, shortenings, soups, and spreads (Bauernfeind et al. 1958; Bunnell et al. 1958; Garnett et al. 2003;

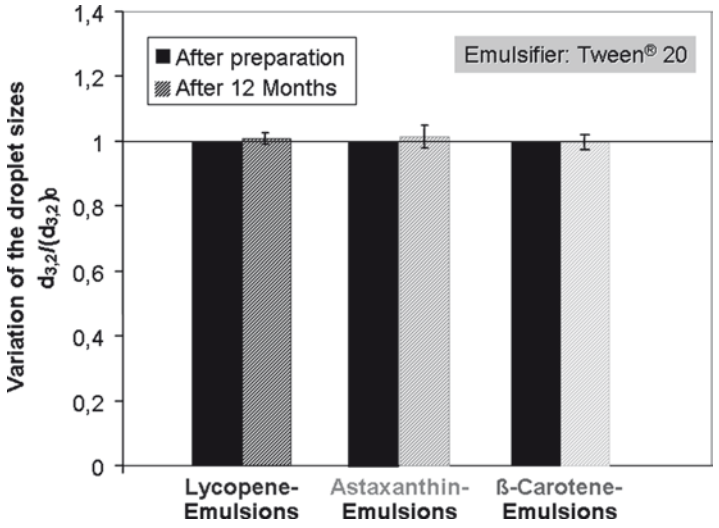

Fig. 8.22 Emulsion stability: Variation of emulsion droplet size (sauter diameter) over 1 year storage time. $d_{3,2o}$ = initial sauter diameter

Leuenberger 2007; Manz 1967; Santipanichwong and Suphantharika 2007; Upritchard et al. 2003). When carotenoid emulsion-based formulations are mixed with products as foods, they have to remain stable even under chemical and physical conditions changed.

The kinetics of carotenoid degradation follows a first-order kinetic model (Henry et al. 1998; Ax et al. 2003). Chemical stability of lycopene-loaded O/W emulsions is given in Fig. 8.24, as an example. The carotenoid formulations were diluted in three different products: skimmed milk, orange juice, and water (control). Three different emulsifiers were used in these investigations. It was found that lycopene stability strongly depends on the food system (Fig. 8.25). In orange juice (pH < 4.5), the lycopene was particularly stable, whereas in milk (pH ≅ 6.6) and water (pH 7), respectively, lycopene concentration decreased quite fast. Stability of lycopene did not depend on the emulsifier type. Addition of α-tocopherol to the dispersed oil phase, however, allowed for prolonged lycopene stability in all three food systems (Fig. 8.26). Antioxidants can be used to protect O/W emulsions against degradation over a long period of time. As can be seen in Fig. 8.27, coalescence of oil droplets was not observed in any of the systems investigated over 3 weeks (Ribeiro et al. 2003).

O/W emulsions containing lycopene and vitamin C, and stabilized by Tween® 20 was also applied in full- and low-fat yogurt, 3.8% and 1.5% fat, respectively. The initial concentration of lycopene in each dairy product was 10 mg/L. It remains stable for 3 weeks in the full-fat yogurt, however, approximately 20% of lycopene degradation could be observed in the low fat one over the same storage time.

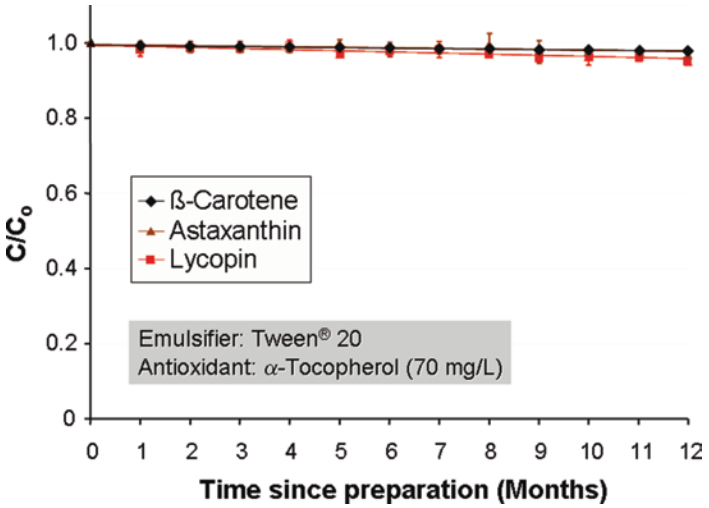

Fig. 8.23 Carotenoid stability in O/W nanoemulsions: Concentration of different carotenoids in nanoemulsions as a function of storage time and α-tocopherol concentration

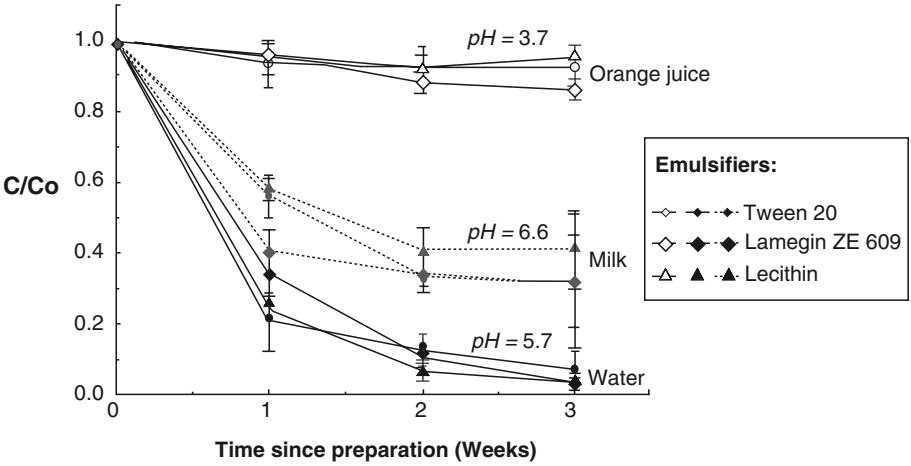

Fig. 8.24 Influence of food matrix on the chemical stability of lycopene without α-tocopherol. C = concentration and C_0 = initial concentration. The *bars* represent standard deviations (Ribeiro et al. 2003) (Courtesy of Wiley-Blackwell Publishers)

By combining emulsifiers a positive influence on carotenoid stability in nanoemulsion-based formulations was found. Ribeiro et al. (2006b) have shown decreased lycopene and astaxanthin degradation in O/W emulsions stabilized with a combination of emulsifiers, especially for a combination of a whey protein isolate and a sugar ester, compared to emulsions prepared with a single emulsifier.

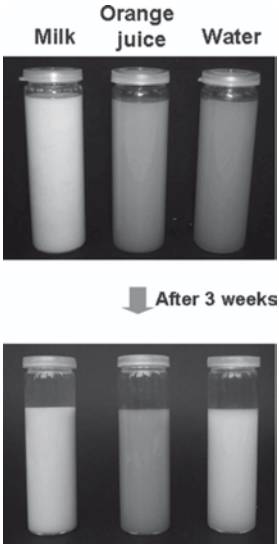

Fig. 8.25 Appearance of the three model systems after incorporation of O/W emulsion containing lycopene and after 3-weeks storage. Emulsifier: Tween® 20

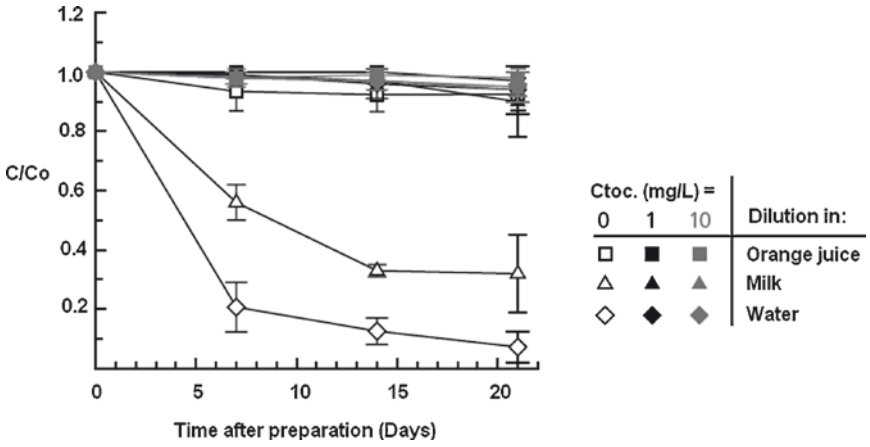

Fig. 8.26 Influence of tocopherol content and food matrix on lycopene stability in O/W emulsions during storage at 4°C. C = concentration and C_0 = initial concentration. The *bars* represent standard deviations (Ribeiro et al. 2003)

Physical stability of carotenoid loaded nanoemulsions includes stability against droplet coalescence and flocculation or aggregation. Droplets can be stabilized against these by steric hindrance of long emulsifier molecules adsorbed at the interfaces and/or by electrical charges. The droplets zeta-potential is a measure of stabilizing

electrostatic interaction forces between the droplets. The droplet zeta-potential of O/W emulsions containing lycopene, prepared with combination of whey protein isolates (BiPro®, BioZate®), with either Tween® 20 or sucrose laurate (L-1695), two non-ionic emulsifiers, as a function of pH is shown in Fig. 8.28. At the lowest pH the droplets had a relatively high positive zeta-potential because the pH was below the isoelectric point of the whey proteins. When the pH was increased, the magnitude of the positive charge on the droplets decreased. Eventually the zeta-potential of the droplets became zero (isoelectric point at a pH between 4 and 5). A further increase in pH caused the droplets to gain a net negative charge.

Droplet aggregation of the O/W emulsions was observed to occur around the isoelectric point (pH 4–5). Figure 8.29 shows the lycopene-loaded O/W emulsions in different pH ranges (2–9) 4 and 24 h after changing the pH, respectively. Droplet agglomeration could be observed after 24 h in samples at pH 4. Droplet flocculation/aggregation caused a significant increase in the creaming of the droplets in the emulsions. Aggregation and creaming stability of whey protein stabilized emulsions has been previously found to depend strongly on pH (Phillips et al. 1994). Emulsion droplets were highly susceptible to aggregation near the isoelectric point of the whey proteins because of the relatively low electrostatic repulsion between the droplets. Samples prepared with astaxanthin showed the same physical and chemical stability behavior. The droplet size of the emulsion containing lycopene and using the emulsifiers L-1695 and BioZate® is shown in the Fig. 8.30 in a pH range between 2 and 9. The measurements were carried out 4 h after changing the pH. It can be seen that droplet sizes increased around the isoelectric point, which means that the emulsions were unstable at this pH due to droplet agglomeration.

8.3.2 Stability of Carotenoid Formulations During Passage Through the In Vitro Gastro-Intestinal Tract

The in vivo efficacy of formulations containing functional ingredients depends on the one hand, on the stability of the emulsions during processing and storage, and on the other hand, on their behavior while passing through the gastro-intestinal tract. After oral ingestion of the emulsions, the chemical and enzymatic conditions in the stomach and small intestine can lead to extensive chemical and physical changes in the emulsion system and thereby influence the absorption of the active substance at the area of resorption. To simulate the influence of the physiological conditions during digestion, a model system (Fig. 8.31) was used. This model consists of a stomach section and a small-intestine compartment. Simulated gastric, biliary, and pancreatic secretions are introduced into the corresponding compartment. The pH is monitored and controlled to the specific values for the stomach and small intestine. The temperature is kept at 37°C. As a basis for the preparation of the digestive secretions the systems described by Minekus (1998) and the German Institute for Standardization (DIN 19738 2004) (see Table 8.1)

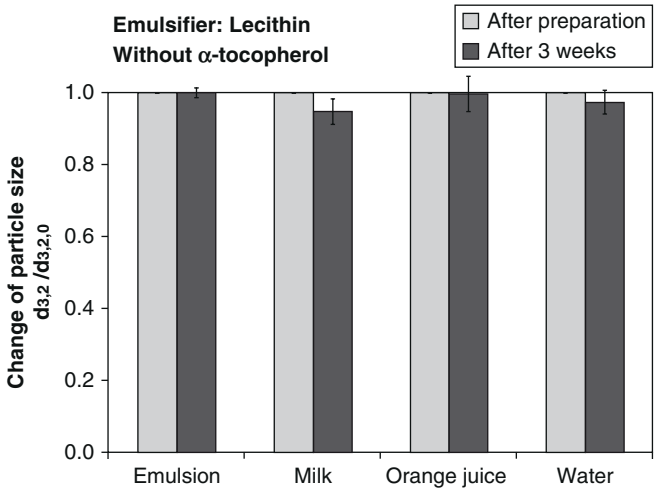

Fig. 8.27 Changes in the sauter diameter ($d_{3,2}$) over 3 weeks. $d_{3,2o}$ = initial sauter diameter. The *bars* represent standard deviations

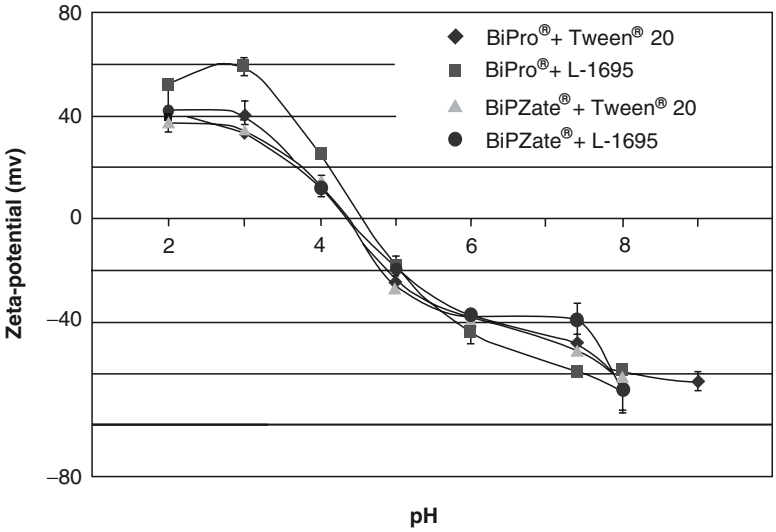

Fig. 8.28 Droplet zeta-potential as function of the pH for O/W emulsions containing lycopene

were adopted. To study the effect of digestion on the stability of different formulations, two test samples were investigated: an emulsion stabilized by Tween® 20 and a sample of liposomes composed of Phospholipon 80®, both containing lycopene (Ax 2003).

As an indicator of the stability of the formulations against aggregation or coalescence, particle size distributions and zeta potential were measured. For carotenoid emulsions, the zeta potential changed from −35 mV at the beginning to about −20 mV after stomach incubation. The zeta potential of liposomes increased from −60 mV to −20 mV. In Fig. 8.32, particle size distributions of untreated carotenoid emulsions and liposomes as well as formulations after incubation are shown. No significant change in droplet size distribution after processing in the stomach was observed in any of the investigated carrier systems.

Under the conditions in the small intestine, dispersed lipid systems were disintegrated under the influence of pancreatic lipase enzymes. Addition of pancreatic lipase to the emulsion led, as a result of lipolysis, to a prompt decline in pH. The appearance of the emulsions changed from opaque to translucent, they darkened in color, and viscosity changes occurred. All these observations indicate that

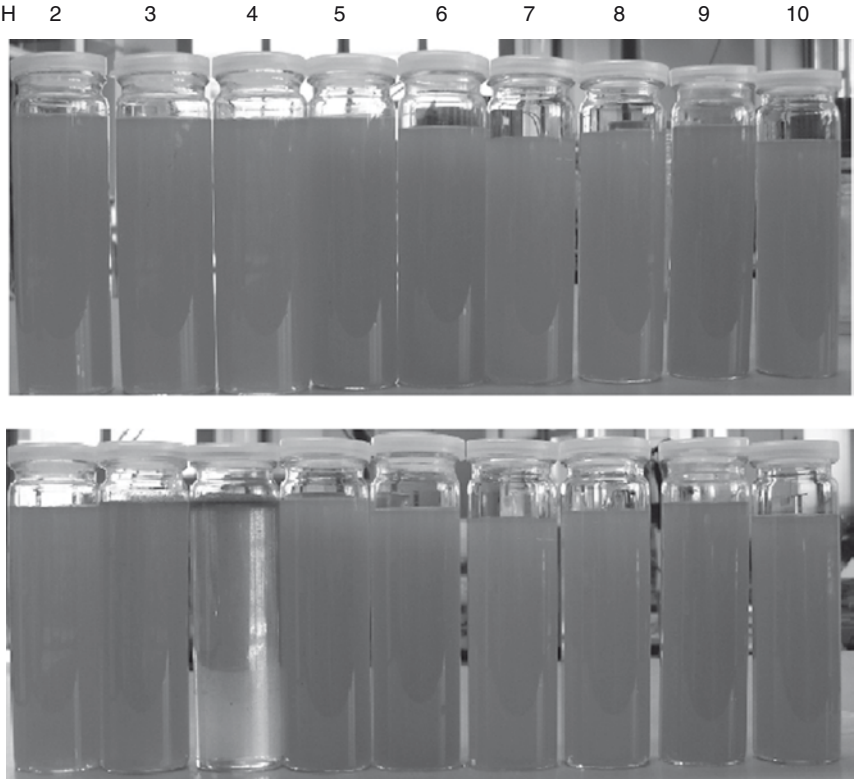

Fig. 8.29 Appearance of diluted astaxanthin-loaded O/W emulsions stabilized by sucrose laurate (L-1695) + BioZate® after changing the pH and over 24 h. In both figures, the emulsion on the left is the emulsion with pH 2.0. The pH increases successively from left to right, with the emulsion with pH 9.0 on the right

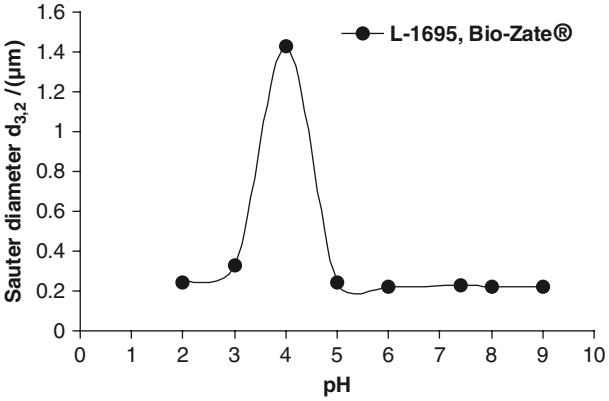

Fig. 8.30 Sauter diameter of the emulsion containing lycopene stabilized by sucrose laurate and BioZate® in different pH

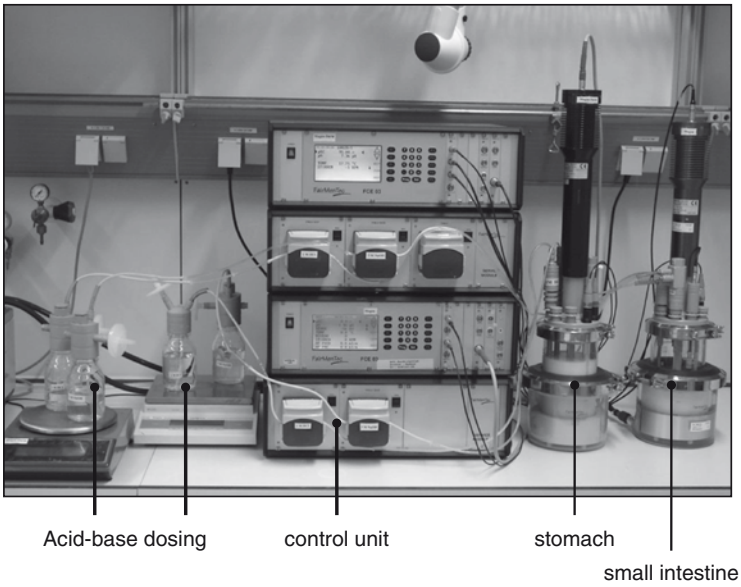

Fig. 8.31 In vitro gastrointestinal tract model

the dispersed oil droplets were destroyed. Liposome incubation under the conditions of the upper intestine resulted in a comparatively slow reaction. The liposome formulation became more transparent and acquired a more viscous, gel-like consistency.

Table 8.1 Modeling of the gastrointestinal conditions

	Stomach	Intestine
Temperature (°C)	37	37
pH	1–5	7–9
Dilution (dispersion:secretion)	3:5	1:4
Electrolyte	NaCl; KCl; KH_2PO4	NaCl; KCl; $CaCl_2 \cdot 2H_2O$; Bile acid
Enzyme	Pepsin	Pancreatin
Incubation time (h)	3	5

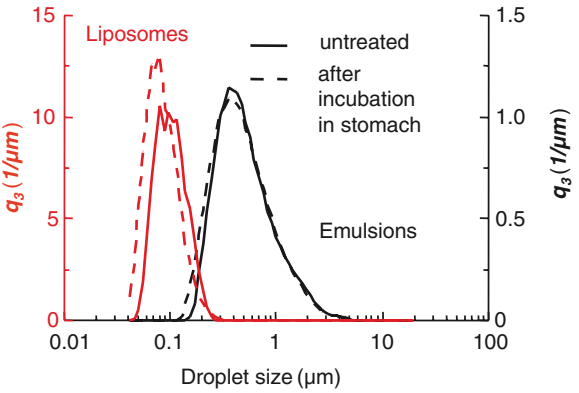

Fig. 8.32 Influence of stomach digestion on carotenoid emulsion and liposome (Ax 2003) (Courtesy of Shaker Verlag)

8.3.3 Bioavailability and Cellular Uptake of Carotenoids

Intestinal carotenoid absorption in human depends on a number of factors such as the carotenoid species, molecular linkage, the amount of carotenoids consumed in a meal, the matrix in which the carotenoid is incorporated, effectors of absorption, the nutrient status of the host, genetic factors, host-related factors, and the interaction of these factors (Castenmiller and West 1998; Parada and Aguilera 2007).

This complexity is responsible for the large inter-individual variability in bioavailability and plasma concentration of carotenoids observed in many intervention studies.

Fruit and vegetables and their products are the major dietary source of carotenoids. In plant tissue, carotenoids are localized in cellular plastids, where carotenoids are associated with light-harvesting complexes or crystalline structures. Carotenoids are generally insoluble in water. In the small intestine carotenoids are

extracted from the food matrix, solubilized in bile acid micelles and absorbed by the enterocytes. The intracellular localization, form of carotenoid (free, protein-bound, crystalline), and intactness of the plant cellular matrix are important determinants of carotenoid bioavailability. There are pronounced differences in the relative bioavailability of carotenoids from different fruit and vegetables and their products, for example, the consumption of fruits resulted in plasma concentrations of β-carotene which were six times higher than those found after consumption of green leafy vegetables (de Pee et al. 1998). Disruption of the plant tissue matrix by mechanical homogenization or thermal treatment enhances the bioavailability of carotenoids (Gärtner et al. 1997; Porrini et al. 1998). Physico-chemical properties of carotenoid species are also important for bioavailability. The bioavailability of lutein, which is more polar than β-carotene, is fivefold to ninefold higher than that of β-carotene from vegetables such as spinach. Dietary fat (3–5 g per meal) and endogenous emulsifiers (bile acids) are necessary to incorporate carotenoids released from the food matrix into mixed micelles. It appears that 3–5 g of fat is sufficient for carotenoid absorption (van het Hof et al. 2000). All these factors which depend on food, food treatment and composition, secretion of digestive enzymes and bile acids into the small intestine make it difficult to predict the plasma concentration (relative bioavailability) for an individual person on the basis of his/her fruit and vegetable consumption. The same amount of fruit and vegetables can result in plasma concentrations with a difference of one order of magnitude. Carotenoid formulations, such as emulsions, supply carotenoids, fat, and emulsifiers in a matrix from which carotenoids can be directly taken up by enterocytes or easily incorporated into mixed micelles. Such formulations could allow better control of the bioavailability of carotenoids.

In vitro cell culture models make it possible to investigate the uptake of carotenoids by intestinal cells and to avoid the effects of some factors which are difficult to control: efficacy of food matrix disruption, solubilization of carotenoids, and incorporation into mixed micelles (Briviba et al. 2001).

There are several ways to solubilize carotenoids in water in order to investigate the cellular uptake or effects of carotenoids in cell culture model systems. Carotenoids dissolve readily in apolar solvents such as chloroform, acetone, or tetrathydrofuran (THF). THF is often used to solubilize carotenoids in water. A final concentration of 0.5% (v/v) of tetrahydrofuran in water is high enough to solubilize most of carotenoids at concentrations of up to 10 μM (Bertram et al. 1991). This THF concentration usually did not affect viability in most cell lines. Cells incubated with astaxanthin delivered in THF at the same concentration as β-carotene (10 μM) showed about a 50-fold higher amount cellular amount of astaxanthin, as detected by HPLC. These observations were also confirmed by confocal resonance Raman microspectroscopy image analysis – a strong Raman signal of astaxanthin but no detectable signal of β-carotene was found in cells (Briviba et al. 2006).

Incubation of HT 29 cells with O/W emulsions prepared using Tween® 20 as an emulsifier and loaded with 10 μM β-carotene or astaxanthin resulted in an increase in the cellular concentration of carotenoids. A slight dependence of the cellular carotenoid concentration on droplet size was observed (Fig. 8.33). The cellular uptake of

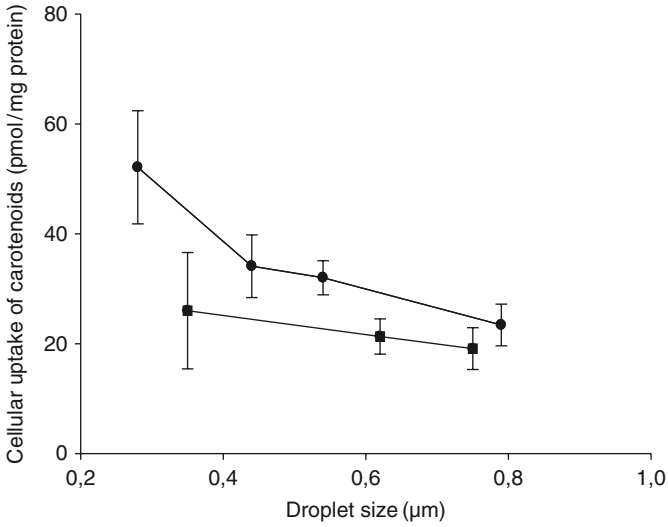

Fig. 8.33 Effect of droplet size of O/W emulsions loaded with carotenoids on cellular uptake of astaxanthin (*squares*) or β-carotene (*circles*)

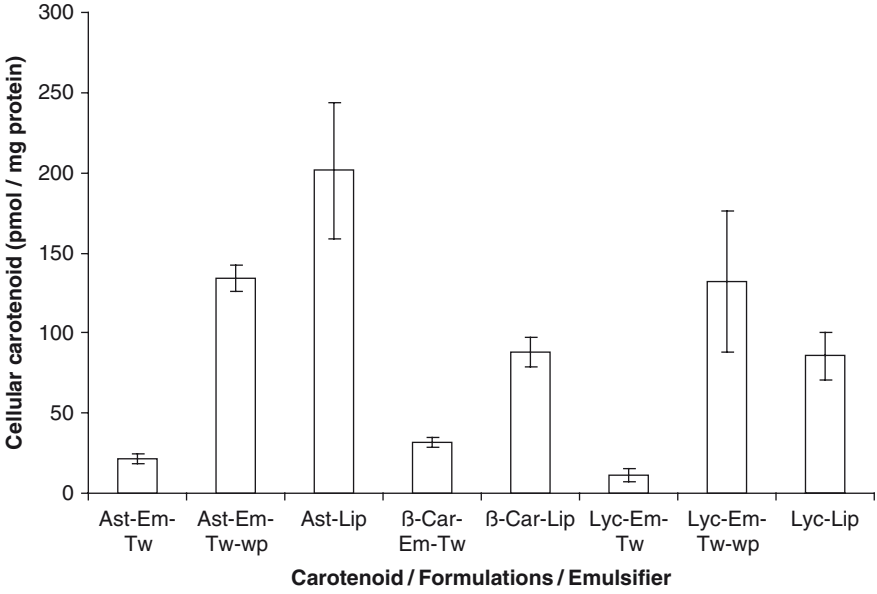

Fig. 8.34 Effect of carrier type and carotenoid species on cellular uptake

astaxanthin and β-carotene from O/W emulsions was comparable. The cellular uptake of carotenoids from emulsions depends on the emulsifiers used. Whey proteins combined with Tween® 20 or sucrose laurate significantly increased carotenoid (astaxanthin, lycopene) uptake from emulsions (Ribeiro et al. 2004; Ribeiro et al. 2006b). Effective cellular uptake of carotenoids (astaxanthin, β-carotene, lycopene) from carotenoid-enriched liposomes prepared from phosphatidylcholine was observed. Carotenoid uptake from liposomes was more effective than from Tween® 20 emulsions, but comparable to or lower than that derived from whey protein/Tween® 20 emulsions (Fig. 8.34). Thus, the uptake of carotenoids by the intestinal cells depends on carotenoid, species, carrier type (emulsions, liposomes), and the chemical ingredients of the formulation (e.g., whey proteins). HT 29 cells line were treated with cell culture medium supplemented with either carotenoid-enriched O/W emulsions with emulsifier Tween® 20 (Em-Tw) or emulsions with a combination of two emulsifiers Tween® 20 and whey protein isolate (Em-Tw-wp) or liposomes (Lip). The whey protein isolate used was BioZate®. Cell culture medium was replaced daily. Cellular levels of carotenoids were estimated after incubation for 72 h. Carotenoids were extracted and determined by reversed-phase HPLC.

8.4 Final Considerations

This chapter has shown a wide range of process to encapsulate carotenoids. Optimal powder or liquid carotenoid formulations allow the production of a physically/chemically stable and bioavailable structure after their preparation, storage, and application in food systems. These requirements are very important for delivering the carotenoid into the gastro-intestinal tract, and later for being absorbed. Encapsulation of carotenoids may meet these requirements. Other criteria to select the right type of encapsulates might be food grade processes, easiness of production, application in dry or liquid products, clarity/transparence of liquid product, large volume production, costs, IPR situation, etc. The ideal encapsulation process and type of formulation will depend on specific application, market requirement, and current regulations.

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