

# Highly Concentrated Emulsions Containing Carotenoids

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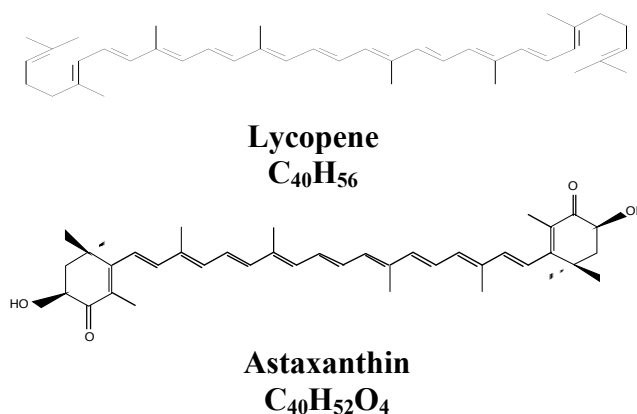
In this work, O/W emulsions containing lycopene or astaxanthin in two different disperse phase fractions were produced by high pressure homogenization. In addition, Tween 20<sup>®</sup> was used as emulsifier and Xanthan as stabilizer. The aqueous phases of the emulsions were separated by centrifugation. By this method, dispersed phase fractions of up to 94 % could be obtained. Emulsions with a Sauter diameter of about 0.34 ( $\varphi_o = 0.2$ ) and 0.7  $\mu\text{m}$  ( $\varphi_o = 0.5$ ) were obtained. The carotenoid concentrations, the physical stability of the droplets and the rheological properties of the initial and the highly concentrated emulsions were determined.

## 1 Introduction

Carotenoids can be found in different kinds of fruits and vegetables as well as in fish. Studies relate carotenoids with protection against some cardiovascular diseases and some kinds of cancer because of their high antioxidant potential and their capacity of inactivating free radicals [1].

Lycopene is an apolar, acyclic carotenoid found mainly in tomatoes, tomato products and lycopene-carrots. Lycopene shows a very high quenching rate of reactive singlet oxygen [2] and is considered the carotenoid with the strongest antioxidative effect. High lycopene concentrations in blood lead to a diminished risk for heart attack by means of decreasing the LDL-cholesterol level. In addition, preliminary research suggests that lycopene may reduce the risk of some cancers such as prostate, lung or stomach cancer [1].

Astaxanthin has two cyclic end groups with polar groups. It occurs in maritime environment and is responsible for the reddish colour of salmons, crabs, lobsters and fish eggs. The colouring and healthiness promoting effect of many maritime organisms are based on the absorption of astaxanthin through their nutrient chain. The structure of astaxanthin is similar to other healthy carotenoids as lutein and zeaxanthin. Additionally, it has a higher antioxidant effect and strengthens the immune system [3]. The chemical structures of lycopene and astaxanthin are shown in figure 1.



**Figure 1.** Chemical structure of lycopene and astaxanthin

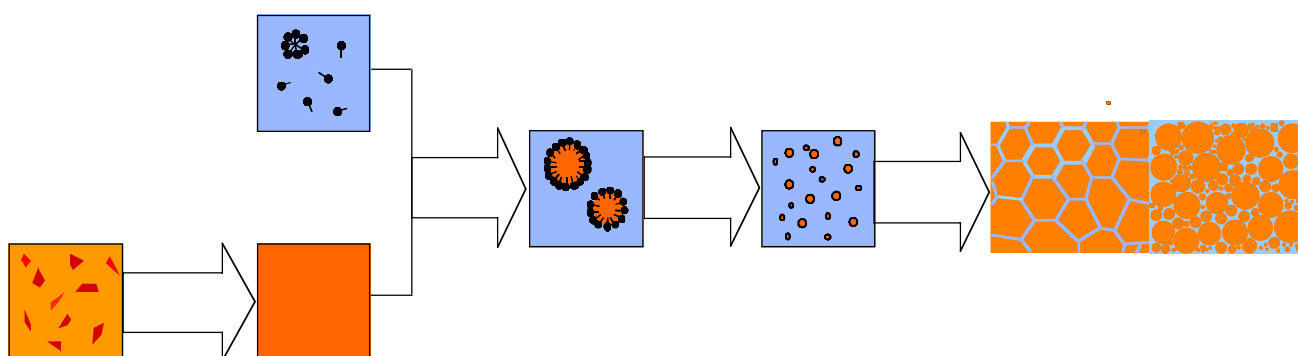
Carotenoids are insoluble in water and soluble in oil only to a very small extent at room temperature. Therefore, their crystalline forms are considered poorly bioavailable. For an ideal absorption, the carotenoids should be dissolved in oil [4]. Oil-in-water (O/W) emulsions containing carotenoids are a water-dispersible carotenoid formulation that may provide good bioavailability. Such systems can be produced after dissolving carotenoids in hot oil, which is consequently emulsified into an aqueous phase. By high pressure homogenization, fine dispersed droplets can be produced [5]. When droplet sizes of O/W emulsions are below 1  $\mu\text{m}$ , carotenoids can be kept soluted in oil in supersaturated concentrations [6, 7].

The emulsion can be centrifuged in order to raise its disperse phase fraction and consequently its carotenoid concentration. In this way, a system is produced with a stable disperse oil phase corresponding to the gaseous phase in a polyhedral or spherical foam. When the volume fraction of the dispersed phase exceeds that of the closely-packed-spheres configuration,  $\varphi_p = 0.7405$  for monodisperse emulsions, the droplets are deformed against their neighbors. They remain separated by thin films of continuous phase that are stabilized against rupture by absorbed layers of emulsifier or polymers. The further the dispersed phase fraction is increased, the more pronounced is the polyhedral shape of the droplets [8].

An emulsion can be considered as highly concentrated when the disperse phase fraction is greater than 90%. Formulation of lipophilic substances, e.g. carotenoids, is possible in a highly concentrated emulsion if the water content of the O/W emulsions decreases. This emulsion is dispersible in aqueous systems and can be applied in foods, e.g. fruit juices, milk products, etc [9].

## 2 Material and Methods

Figure 2 shows the scheme of the process for producing O/W emulsions enriched with carotenoids [5]. A fractionated palm-oil was used (Bergabest MCT, Schumann und Sohn, Karlsruhe). The oil contents of the initial emulsions were 20 % and 50 %. Crystalline Lycopene and Astaxanthin had a purity of about 95 % and 80 %, respectively (BASF AG, Ludwigshafen). Polyoxyethylene (20)-Sorbitan-Monolaurat (E 432) (Tween 20<sup>®</sup>, Carl Roth GmbH & Co. KG, Karlsruhe) was used as emulsifier at 1 wt.% referring to the whole emulsion. Emulsions were stabilized with Xanthan (0.25 wt.%, E 415, Carl Roth GmbH & Co. KG, Karlsruhe).  $\alpha$ -Tocopherol (E 306, Carl Roth GmbH & Co. KG, Karlsruhe) was added to the oil phase as antioxidant at a concentration of 10 mg/L.



**Figure 2.** Principle of producing of highly concentrated carotenoid-loaded O/W emulsions.

In order to increase the disperse phase fraction of the O/W emulsions, creaming of the disperse phase was induced by centrifugal acceleration of 8000 g and 38000 g for two, six and ten hours in order to separate and remove the supernatant continuous phase (Sorvall<sup>®</sup> Centrifuge Model RC-5C, Du Pont Company, Delaware) [10]. To determine the concentration of astaxanthin and Lycopene, they were extracted with n-Hexane. The concentration of carotenoids was measured spectrophotometrically (U2000, Hitachi Europe GmbH, Düsseldorf) [11, 12]. The droplet size distribution was analyzed by laser diffraction in combination with polarization intensity differential scattering (PIDS) technology (LS 230, Beckman-Coulter GmbH, Krefeld).

The rheological measurements were carried out with a rheometer under controlled stress regime (RS600, Thermo Electron GmbH, Karlsruhe). The non-Newtonian viscous behavior (steady shear) of the emulsions was determined in a double concentric cylinder geometry (DG41) with a

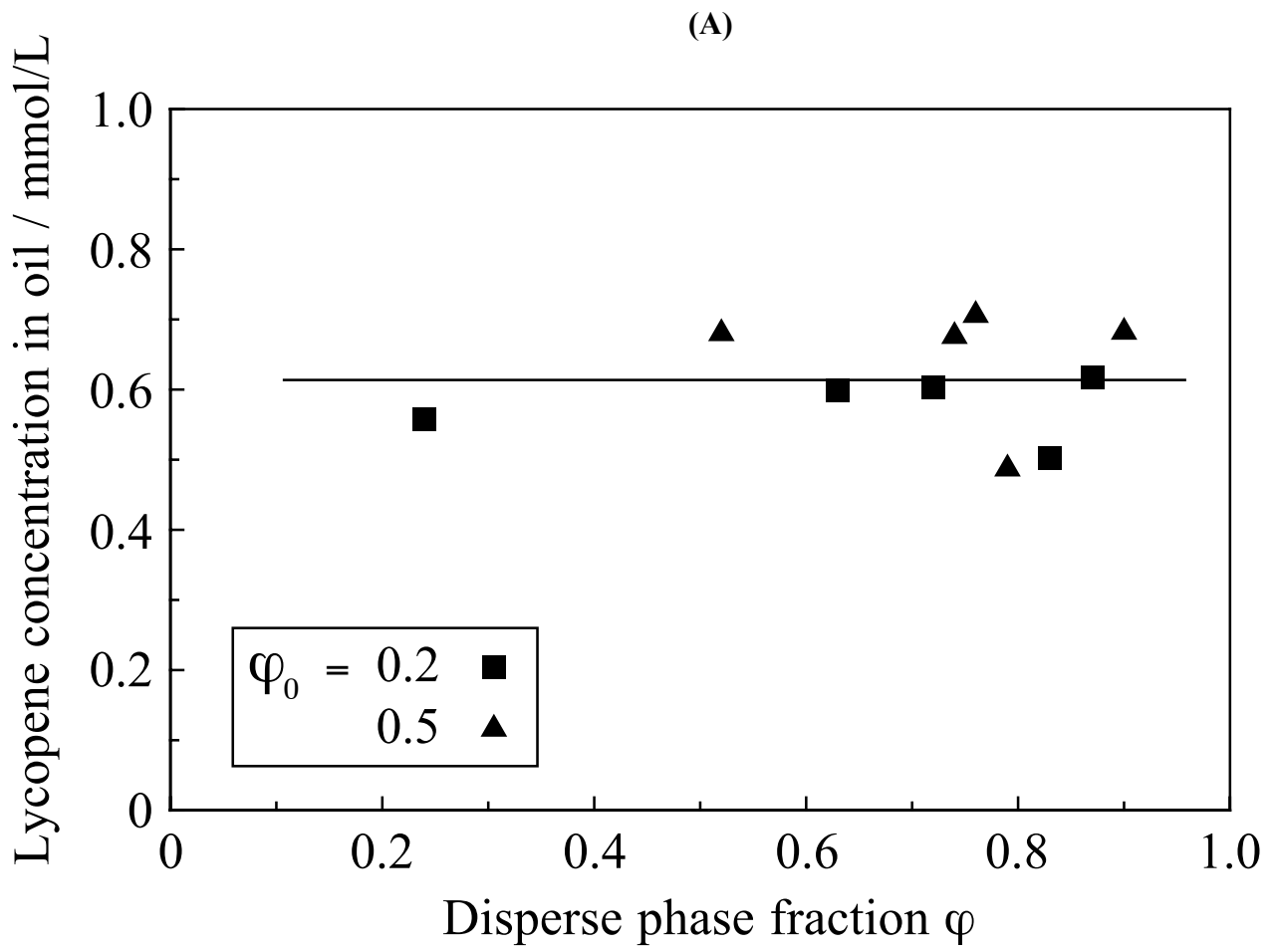
stationary measurement. The shear stress was increased exponentially from 0.01 to 10.0 Pa over 3 minutes. For highly concentrated emulsions, a parallel plate geometry (oscillatory measurements) with a diameter of 20 mm (PP20-Ti) and a gap of 1.0 mm between the two surfaces was used. The shear stress varied from 10 to 10000 Pa under constant frequency of 1 Hz. All measurements were carried out at a constant temperature of 25 °C.

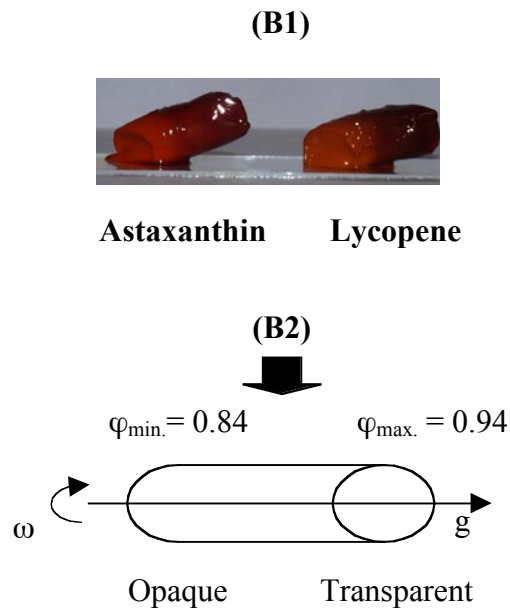
### **3 Results and Discussion**

#### **3.1 Chemical Stability of Carotenoids**

The fine dispersed emulsions containing carotenoids had a maximum carotenoid concentration of 3 mmol/L. In the highly concentrated emulsions, a maximum carotenoid concentration of 7 mmol/L was obtained. Addition of  $\alpha$ -Tocopherol provided a good stability of lycopene and astaxanthin during the experiments (Figure 3A).

An emulsion can be significantly concentrated under the influence of a centrifugal force. The droplets are forced to deform, i.e., they flatten in those areas where they make contact and assume a polyhedral shape with rounded corners and edges [13]. After centrifugation and removal of the supernatant continuous phase, emulsions turn into a rigid structure with high oil content are elastic (gel-like) and transparent (Figure 3B1 and B2) [10].



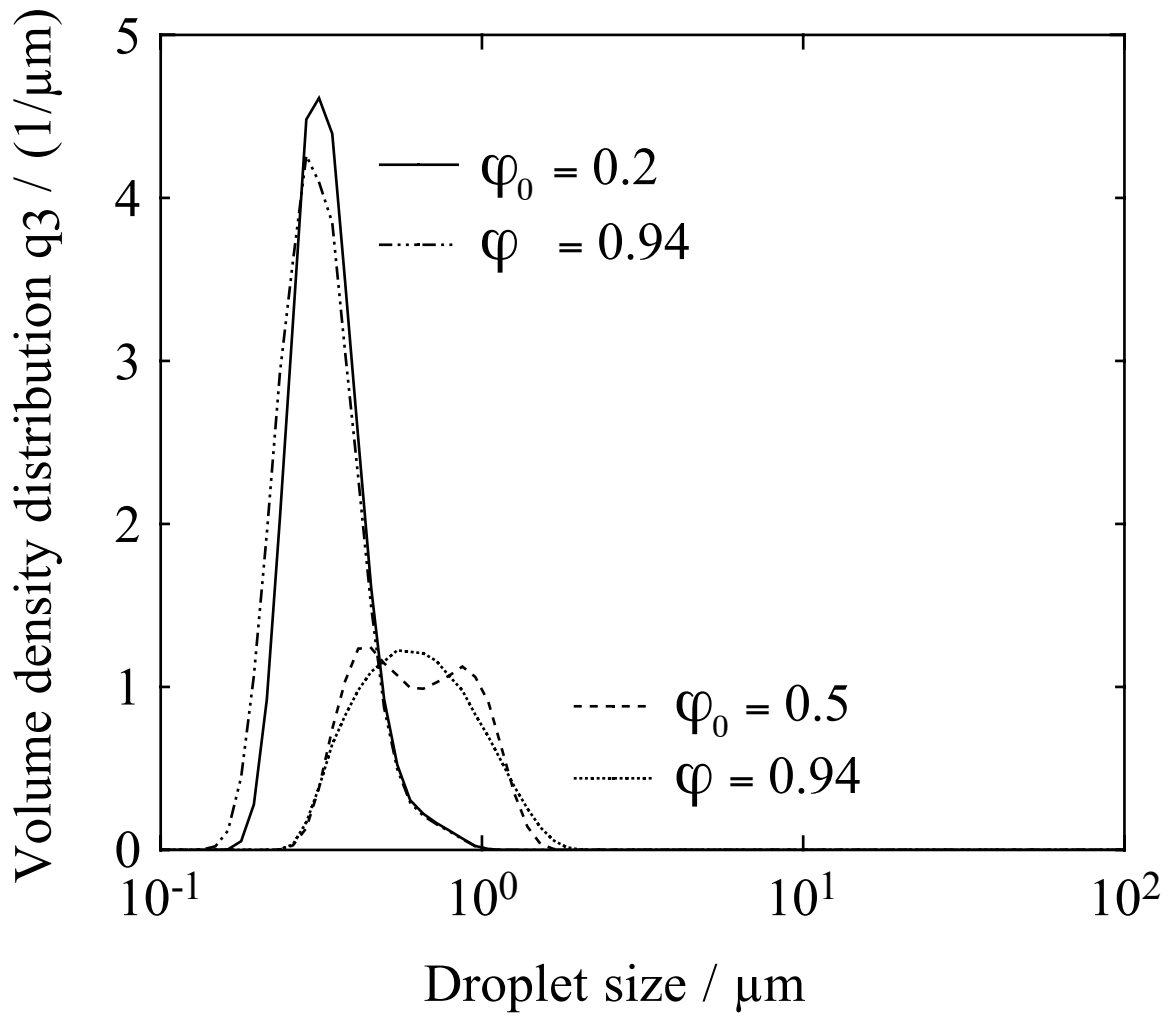


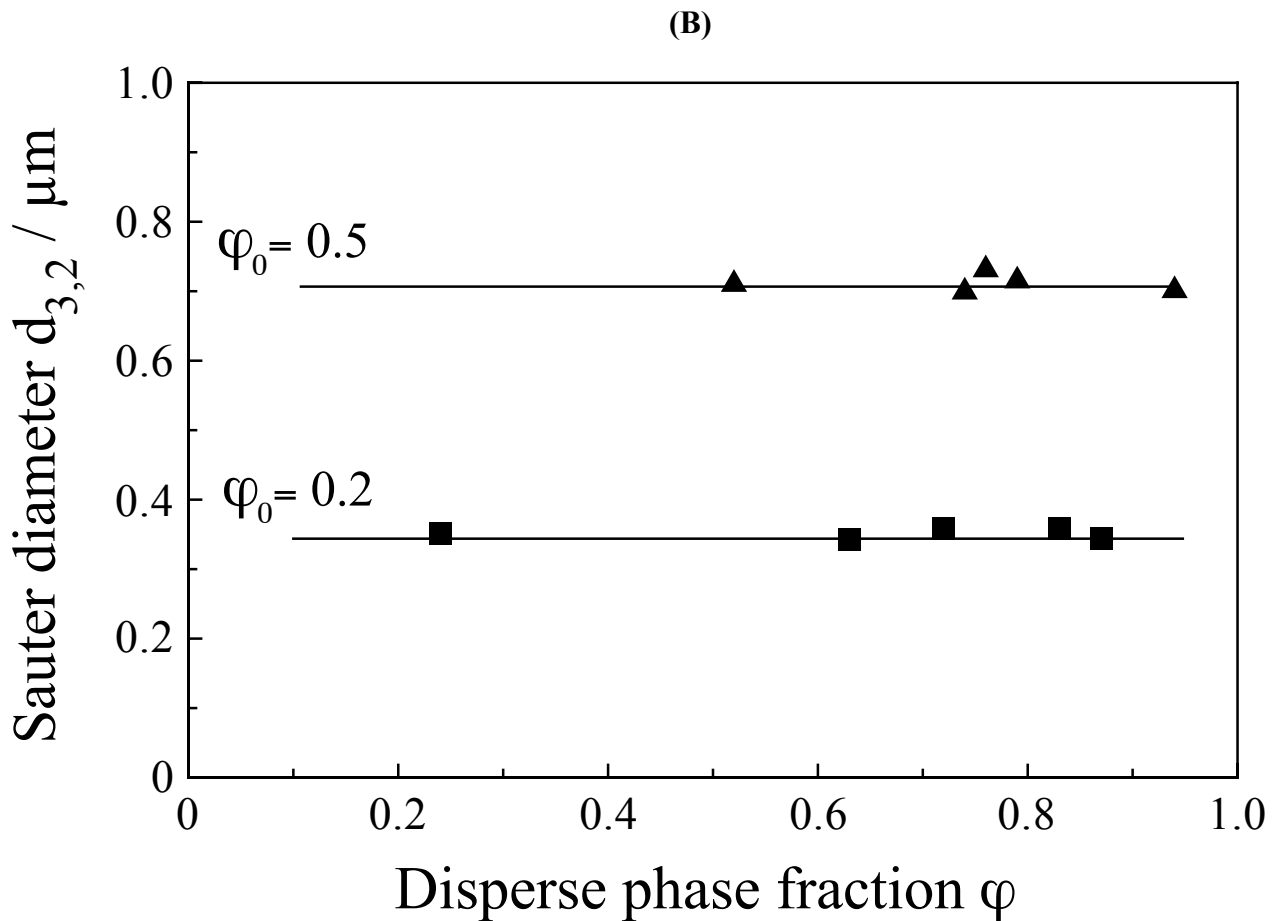
**Figure 3.** Lycopene concentration in O/W emulsions with different disperse phase fractions (A), appearance of the highly concentrated lycopene and astaxanthin O/W emulsion (B1) and sketch of variation of the disperse phase fraction of the samples after centrifugation (B2).

### 3.2 Physical Stability of the Droplets

An important characteristic of emulsions is the droplet size distribution since it has significant influence on many properties. Using centrifugation at high centrifugal accelerations over long time, e.g. 38000 g for 10 hours, a highly dispersed phase fraction of approximately 94 % could be obtained without considerable changes in the droplet size distributions. By separation of the continuous phase of the emulsion containing Tween20<sup>®</sup> as emulsifier and Xanthan as stabilizer, the volume density distribution ( $q_3$ ) remained nearly constant (Figure 4A). The same behavior was observed for the Sauter diameter of the emulsions (Figure 4B).

(A)





**Figure 4.** Droplet size distributions (A) and Sauter diameters (B) of the emulsions.

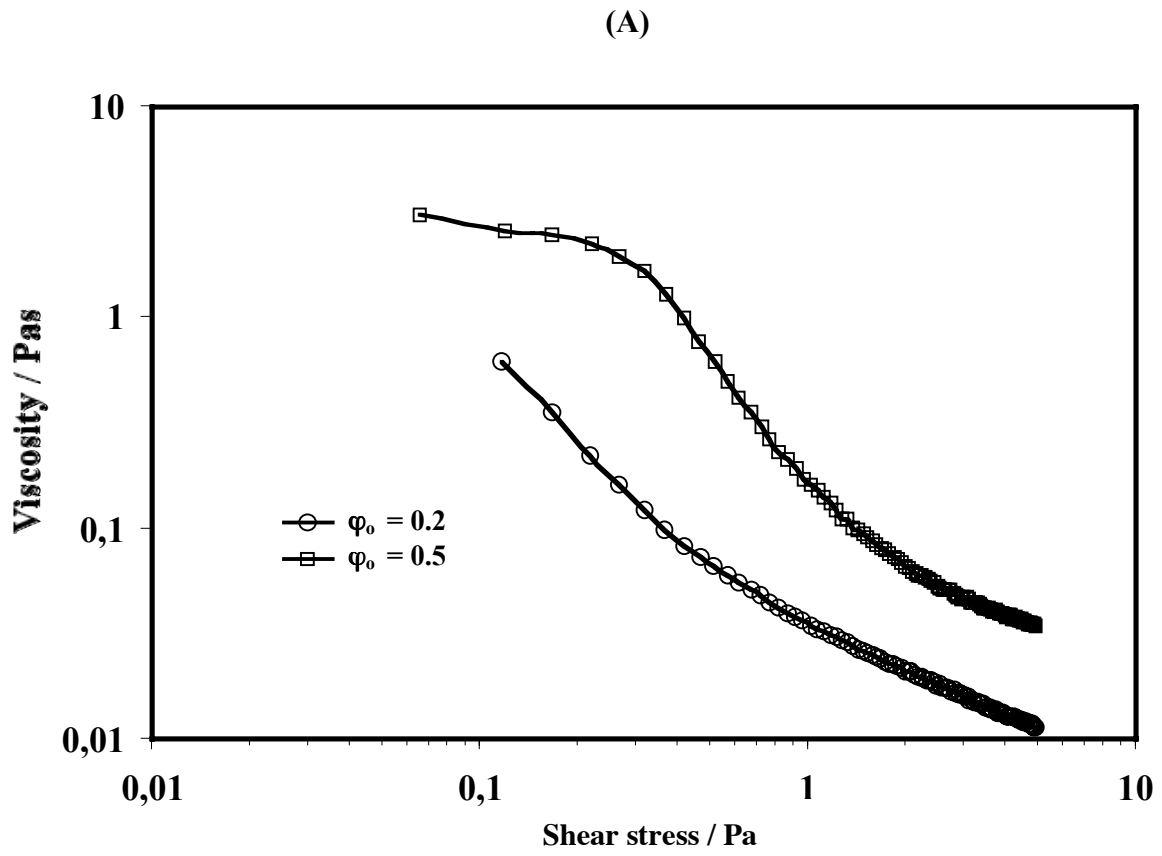
### 3.3 Rheological Properties

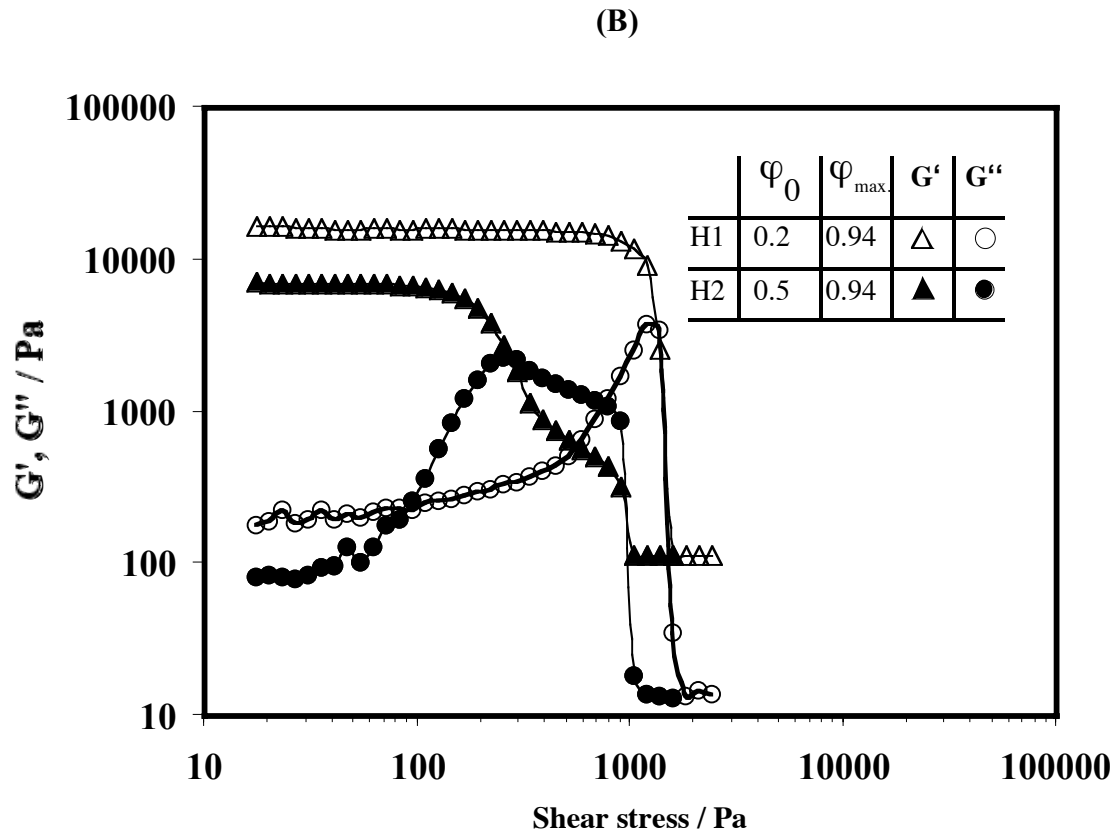
The disperse phase fraction, the viscosity of the continuous and disperse phase, the droplet size, the droplet size distribution and the kind of emulsifier and stabilizer used are important factors for the flow behavior of emulsions.

The viscous behavior of the emulsions containing 20 % and 50 % of dispersed phase are shown in Figure 5A. It can be seen that the viscosity of the emulsions decrease with increasing shear stress. This behavior is characterized as shear-thinning. For initial emulsions, an increase in the disperse phase fraction from 20 % to 50 % increases their viscosity and a structure is formed (Fig. 5A, curve  $\varphi_0 = 0.5$ ). This structure can be macroscopically correlated with the existence of a yield stress. In fact, for low shear stresses values ( $< 0.2$  Pa), the emulsion behaves like a plastic solid with constant viscosity (2.0 Pas).

The measured yield stress in the higher concentrated emulsion suggests the existence of a network between the droplets. The higher the disperse phase fraction, the shorter is the free mean

distance between droplets, yielding a significant increase in the viscosity of the emulsion and changes in its structure.





**Figure 5.** Effect of the disperse phase fraction on the emulsions' flow behavior: viscous ( $\varphi_0 = 0.2$ ); viscoplastic ( $\varphi_0 = 0.5$ ) (A) and viscoelastic ( $\varphi_{max.} = 0.94$ ) emulsions (B).

After centrifugation, higher disperse phase fractions can be obtained. This increase changes the rheological properties of the emulsions from a viscous ( $\varphi_0 = 0.20$ ) and viscoplastic ( $\varphi_0 = 0.50$ ) to a viscoelastic response ( $\varphi_{max.} = 0.94$ ). Thereby, the shear stress range increased from 10 Pa to >1000 Pa (curve H1 and H2, Fig. 5B) and both emulsions revealed a linear viscoelastic behavior. The shear storage modulus magnitudes ( $G'$ ) were 7000 Pa and 15800 Pa for H2 and H1, respectively.

The end of the linear viscoelastic regime marks the beginning of the collapse of the network between the droplets. This can be observed at a critical shear stress  $\tau_{crit.}$  of 130 Pa for the emulsion H2 ( $\varphi_0 = 0.50$ ) and at a higher stress for the emulsion H1 ( $\varphi_0 = 0.2$ ) with  $\tau_{crit.} = 800$  Pa. The droplet network for both emulsions was completely destroyed at shear stresses higher than 1000 Pa.

As can be seen, the collapse of the emulsion structures occurs in different ways. For the emulsion H1, it takes place abruptly in a narrow shear stress range ( $800 \text{ Pa} < \tau < 1842 \text{ Pa}$ );  $\tau_{max.}$  was approximately two times higher than  $\tau_{crit.}$  and the shear storage modulus  $G'$  was higher than the shear loss modulus  $G''$  in the entire measuring range. For the emulsion H2, in contrast to H1, the breakdown of the structure occurs in two steps and in a wider shear stress range

(130 Pa <  $\tau$  < 1207 Pa);  $\tau_{\max.}$  was about nine times higher than  $\tau_{\text{crit.}}$  and a crossover point could be detected at  $\tau_{\text{cop.}} = 276$  Pa ( $G' = G''$ ). For shear stresses above 276 Pa, the measurements revealed that emulsion H2 had a predominant viscous behavior ( $G'' > G'$ ). The first emulsion partition structure collapses between  $\tau_{\text{crit.}} < \tau < \tau_{\text{cop.}}$  and the second one between  $\tau_{\text{cop.}} < \tau < \tau_{\max.}$ . This difference between the emulsions' structures led to distinct stability grades that still have to be interpreted microscopically. Most probably, the different behavior is due to the different droplets size distribution of the two emulsions.

In contrast to the viscous flow behavior of low concentrated emulsions, the droplet size and the droplet size distribution plays a decisive role in the variation of the viscoelastic properties of highly concentrated emulsions.

The emulsion H1 had a smaller Sauter diameter of  $d_{3,2} = 0.34$   $\mu\text{m}$  and a narrower droplet size distribution than the emulsion H2 with  $d_{3,2} = 0.70$   $\mu\text{m}$  (Fig. 4).

With a decrease of the droplet size, the droplets specific surface area and the number of droplets increase, thus providing more interface contacts between droplets. This effect of the difference in the droplet size distribution and in the total number of droplets also applies for the structure of high concentrated emulsions. The possible number of contact points between droplets and/or the extension of contact surfaces between droplets are greater in emulsion H1 than in emulsion H2. As almost all droplets from emulsion H1 have the same size, its final structure is more homogeneous than that of H2. It is known that the magnitude of the stresses required to separate the droplets is proportional to the number of contact surfaces between droplets. As a response to this homogeneity of its structure, the structure of emulsion H1 broke down spontaneously and in a narrow and clear defined shear stress range. The droplet size distribution of emulsion H2 is wider and the resulting structure from this distribution is apparently more heterogeneous than that of emulsion H1. Furthermore, after centrifugation, the droplets' packing density from the restructured emulsion H2 corresponds to a disorder pattern.

The rheologic behavior of highly concentrated emulsions ( $\varphi_{\max.} = 0.94$ ) is strongly dependent on the structural arrangement of the droplets. A more disordered packing structure is reflected in a wider shear stress range that first leads to a structure deformation and then to the structure's collapse. Possibly, in the emulsion H2, there are at least two dominant structures: one that deforms at  $\tau > \tau_{\text{crit.}}$  and another one at  $\tau > \tau_{\text{cop.}}$ .

## 4 Conclusions

To obtain a high dispersed phase fraction and consequently high concentrations of carotenoids in O/W emulsions, centrifugation is a suitable process. Carotenoid enriched O/W

emulsions can be concentrated up to 94 % of dispersed phase without considerable changes in the droplet size distribution. After centrifugation, no coalescence or aggregation of the droplets were observed. Highly concentrated O/W emulsions show a pronounced viscoelastic behavior. The stability of their structures was influenced by the droplet size distribution of the initial emulsions.

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## Symbol

$\varphi_p$	[-]	Packing density
$g$	[m s <sup>-2</sup> ]	Acceleration of gravity
$G'$	[Pa]	Storage modulus
$G''$	[Pa]	Loss modulus
H1	[-]	Emulsion concentrated from 20 % Emulsion
H2	[-]	Emulsion concentrated from 50 % Emulsion
$\varphi$	[-]	Disperse phase concentration
$\varphi_0$	[-]	Initial disperse phase concentration
$\varphi_{min.}$	[-]	Minimal disperse phase concentration
$\varphi_{max.}$	[-]	Maximal disperse phase concentration
$\omega$	[s <sup>-1</sup> ]	Angular speed
$\tau_{crit.}$	[Pa]	Critical shear stress
$\tau_{max.}$	[Pa]	Maximal shear stress
$\tau_{cop.}$	[Pa]	Shear stress on crossover point

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