Protection of sensitive lipids such as oils against oxidation in foods is often a real challenge due to the heterogeneity of the food matrix and interactions of the matrix biopolymers with water present in the surroundings. Water–biopolymer interactions are important with regard to oxidation since oxygen dynamics is linked with water mobility, which in turn depends on the mobility of matrix molecules. Oils sensitive to oxidation can partly be protected by spray-drying of emulsions with oil as the dispersed phase. Spray-dried particles are also useful model systems to study the number of factors affecting oil oxidation in a bio-matrix and to elucidate the potential routes for improved oxidative stability via better control of oxygen dynamics.

Key words: oil, oxidation, diffusion, biomolecules, spray-drying

1. Introduction

Foods incorporated with or originally containing healthy oils or other beneficial lipids are often prone to oxidation, and controlling oxygen dynamics in those systems is an issue of crucial importance. Spray-dried emulsions with unsaturated oil as the dispersed phase originating from e.g. fish or berry seeds are examples of encapsulated oil-systems targeting not only to better technical performance of the oil but also to an improved protection of the oil against oxidation. Spray-dried emulsions are also good model systems to investigate the generic challenge which exists in attempts to control the oxygen transfer in often very heterogeneous processed food matrices. Such a challenge becomes apparent e.g. in processing of oats which is a specific cereal due to its high content of unsaturated oil. As long as the oat grain is intact the oil is stable but milling or other processing always triggers oxidation reactions.

Spray-dried oil-in-water emulsions are powders composed of particles having diameters of about 30 μm. The phases present in a spray-dried powder particle are schematically shown in Fig. 1. Oxygen present in the atmosphere of the powder is a prerequisite for oxidation. To improve the stability of the dispersed oil phase, the challenge is to control oxygen dynamics from the gas to the oil phase (Fig. 1). If the oil-powder is incorporated into a food matrix in order to increase healthiness of the

\[ \text{Gas phase} \quad \text{O}_2 \quad \text{Matrix phase} \quad \text{interface} \quad \text{Lipid phase} \]

\[ \text{O}_2 \text{ free, dry gas phase} \quad \text{Matrix phase} \quad \text{interface} \quad \text{Lipid phase} \]

\[ \text{stored under O}_2 \text{ atmosphere} \quad \text{& variable a}_w \]

\[ \text{optimal packaging} \]

\[ \text{the challenge is to control oxygen transfer from the gas phase to the lipid phase, across matrix+interface} \]

Fig. 1 Schematic description of the phases present in a particle prepared by spray-drying of an oil-in-water emulsion and exposed to air.
food, the powder particles are also exposed to variable water activity, which reflects in the physical properties of the powder matrix. In case the powder is not added to any foods, the simplest solution to inhibit oxidation is to apply a package which creates a dry and oxygen-free atmosphere. Without packaging the challenge is to develop a matrix and/or an interface which could act as an oxygen barrier, i.e. a material with low rate of oxygen transport. In addition to manipulation of the matrix and the interface, stability of the powder may be improved by addition of antioxidants to the oil and/or removing traces of metal ions still present in the oil. The present work, however, does not deal with the oil phase but concerns the matrix and the interface and the potential options to modify these phases towards better stability of the dispersed oil phase against oxidation.

2. Materials and Methods

Materials and methods are briefly described below concerning those results of the present article which have not been published earlier.

2.1 Materials

Amylose used has been purified from potato starch (A0512, Sigma Chemical Co., St. Louis, MO, USA). Na-caseinate was obtained from DMW International (Veghel, the Netherlands), whey protein isolate from Davisco Foods International (Le Sueur, MN, USA) and pectin originating from sugar beet (Dupont Danisco, Kotka, Finland). All reagents used were of analytical grade.

2.2 Methods

2.2.1 Amylose films preparation

Films from amylose were prepared by wetting amylose powder with ethanol and solubilising in water at 5–10% concentration in a pressure cooker with stirring at 130°C. Ethanol was allowed to evaporate at 95°C for 30 min and solution was cast on a PTFE mould and dried in a chamber at 70–80°C and at relative humidity (RH) 50%. The films were stored at RH 97%, and cut to round pieces with the diameter of 13 mm. Thickness was measured with a micrometer, and mass analysed before immersion into a 50 mmol/dm³ KCl solution. The immersion time was 24 hours.

2.2.2 Preparation of emulsions

Oil-in-water emulsions were prepared of rapeseed oil and water (oil content 25 wt%). Emulsifiers were Na-caseinate, whey protein isolate, pectin and combinations of Na-caseinate and pectin at the concentrations of 1 or 1.5%. A ruthenium based oxygen sensitive fluorescent dye was added to the oil (50 μg/g oil) before emulsification. The dye (tris (4,7-diphenyl-1,10-phenanthroline) ruthenium (II) bis (hexafluorophosphate) stock solution was prepared by solubilisation 5 mg of the dye into 5 ml of chloroform. Emulsification was done by applying a pressure homogenizer (Microfluidics M110-Y, Newton, MA, USA) to a pre-homogenised emulsion. Pressure homogenisation was done at 40 psig (5000 bar), and pre-homogenisation was performed using a stirring-type homogeniser for two times 2 minutes at 26 000 rpm (Heidolph Diax 900, Labexchange, Burladingen, Germany).

2.2.3 Water vapour sorption isotherm

To measure water vapour sorption isotherms film was frozen with liquid nitrogen and milled (Fritsch pulversette 14, Fritsch GmbH, Idar-Oberstein, Germany). About 200 mg of pulverized sample was weighed and stored at 25, 35, 45 and 55°C while RH was adjusted to 10–90% in an environmental chamber. The samples were stored at least for three days and weighed again. Initial moisture was measured by weighing prior and after evaporating the moisture at 130°C for 1.5 hours. Based on the initial water content and the mass change during the equilibration the total water content was calculated and expressed as weight percent of water in the sample (wet basis).

2.2.4 Resistance by impedance spectroscopy

Impedance spectroscopy was applied to measure the resistance of the amylose film from which the conductance and finally the diffusion coefficient of KCl were calculated. Impedance spectrum was measured by a frequency response analyser (FRA, Solartron 1250, Solartron Analytical, Hampshire, UK) coupled with an ohmic resistor in the 0.05–65,325 Hz frequency range (Fig. 2). The amplitude of the sinusoidal signal was 10 mV. A piece of KCl filled film was sandwiched between the Ag/AgCl electrodes, which were pressed together with a weight of 1.5 kg. The system was kept in an environmental chamber at least for three days before the measurement, which were done at six temperatures (15–55°C) and at RH range of 55–95%. In most cases film thickness was 300 μm. The resistance (R) was obtained from the measured impedance data (Fig. 2) which in
turn was changed to conductivity using the equation $\kappa = \frac{L}{\Omega A}$, where $L$ is the thickness and $A$ the area of the film. Finally the diffusion coefficient was approximated from equation $D = \frac{RT\kappa}{(2F^2c)}$, where $R$ is the gas constant (8.314 JK$^{-1}$mol$^{-1}$), $T$ temperature, $F$ the Faraday constant (96487 Cmol$^{-1}$) and $c$ the concentration of KCl in the film.

### 2.2.5 Oxygen concentration and diffusion coefficient

Oxygen concentration in emulsion was analysed based on monitoring fluorescent intensity as a function of time with a plate reader (Victor$^2$, Wallac, Turku, Finland) after careful removal of oxygen with the aid of nitrogen gas bubbling for one hour. A reference emulsion was exposed to air to evaluate the baseline. Gas flows were wetted before addition to the emulsion to minimise evaporation. The excitation and emission wavelength was 484 and 615 nm, respectively. The fluorescent decay of the samples (200 μl) was recorded for 15 minutes with 30 s interval. The oxygen concentration ($M_t$) depends on fluorescent intensity ($I$)

$$\frac{M_t}{M_\infty} = \frac{(I_0 - I_I)I_\infty}{(I_0 - I_I)I_I}$$  \hspace{1cm} (1)

In Eq. (1), the subscript “$\infty$” refers to the quantity values at equilibrium, i.e. formally at infinite time, and the subscript “0” to the initial values. The diffusion coefficient $D$ was calculated from two models both based on Fick’s second law for a spherical object. Model 1 was the same as applied by Tikekar et al [1] in which the surface concentration of oxygen was assumed to be constant and the integration was done from the outer surface to the mid of the droplet. Model 2 assumes that, because of the small dimension of the droplet (radius only about 50 nm), thermal motion is capable of mixing its contents ideally and there are no concentration gradients inside the droplet. Changes in the concentration take place solely due to diffusion across the droplet wall; this is known as the pseudo-steady-state assumption. Fick’s diffusion equation was thus integrated only across the interface, from $r = a$ to $r = a + h$. (See Appendix for the full derivation.) The parameters needed were in model 1 the droplet radius ($a$) and in model 2 the radius ($a$) and the thickness ($h$) of the interface. The solved equations for model 1 and model 2 are

$$\frac{M_t}{M_\infty} = 1 - \sum \frac{1}{n^2} \exp \left( -\frac{n^2\pi^2Dt}{a^2} \right), \text{ model 1}$$  \hspace{1cm} (2)

$$\frac{M_t}{M_\infty} = 1 - \exp \left[ -\frac{3Dt}{a} \left( \frac{1}{a} + \frac{1}{h} \right) \right], \text{ model 2}$$  \hspace{1cm} (3)

The diffusion coefficient was calculated by fitting the experimental fluorescent intensity data with the model.
3. RESULTS

3.1 Better performance of spray-dried powder via matrix manipulation

The most important property related with the stability of a solid amorphous matrix is the glass transition temperature ($T_g$) at which the amorphous material changes from rubbery to glassy when lowering the temperature. The glass transition temperature of a biopolymer ($T_g$) is generally affected by the polymer molar mass and the water content. As earlier demonstrated for a common polysaccharide, starch, $T_g$ decreases with decreasing molar mass and increasing water content (Fig. 3) [2,3].

The generally accepted guidance to stabilize an amorphous carbohydrate matrix for preservation applications is to keep it glassy, i.e. to store at temperatures below $T_g$ or to lower the water content until the matrix reaches glassy state [4]. In regard with encapsulation materials of spray-dried oil-in-water emulsions this means that $T_g$ of the matrix material should be maximized. Native starches with high $T_g$, however, cannot be used as such since they are not water soluble. In contrast, their hydrolysates, such as maltodextrins (MD) in combination with an emulsifier and amphiphilic octenylsuccinate derivatives (HiCap) are water soluble at enough high solids and very suitable for matrix materials, and have, therefore, been frequently applied. We have used MD in combination with gum arabic (GA), and HiCap alone as the matrix materials for encapsulating sea buckthorn seed oil, which easily gets rancid if exposed to oxygen due to its high content of linoleic acid [3,5,6]. The performance of the powder materials were compared by plotting the time during which the quality of the oil was good as a function of the deviation of the storage temperature from $T_g$ (Fig. 4), ending up to the conclusion that in HiCap powders the glass transition temperature controlled the oil stability, as well as in MD–GA powders if stored at 20°C (Fig. 4). The MD–GA powders stored at 50°C, however, behaved differently. More recently, as we were investigating milk protein as the matrix material and flax seed oil as the dispersed phase, the performance of the powder was opposite; the oil dispersed in a rubbery–like material was more stable than in a glassy matrix [7].

As recently pointed out by several investigators, the glassy matrix is not totally immobilized in spite of the high viscosity [4]. In glassy carbohydrates translational molecular movement ceases but not all dynamic processes are inhibited. Tromp et al [8] measured diffusion coefficient of water in maltose and glucose syrup and observed that water is mobile also in the glassy state. The activation energy for water diffusion was shown to be independent of the molar mass and water content of the carbohydrate matrix. This indicated that water diffusion was independent of the macroscopic viscosity, i.e. the diffusion coefficient ($D$) and viscosity ($\eta$) were decoupled, and thus diffusion coefficient of water could not be evaluated from the Stokes–Einstein equation, $D = kT / (6\pi\eta a)$. We also obtained similar behaviour when studying KCl diffusion in an amylose film. The diffusion coefficient of KCl was calculated from the amylose film trans. diff. coeff. as a function of water content and compared with that of water. The results showed that KCl diffusion was much slower than water diffusion.

![Fig. 3](image_url)  
Fig. 3 Effect of water content on glass transition temperature of starch and maltodextrin with DE 20, data extracted from Myllärinen et al [2] and Partanen et al [3].

![Fig. 4](image_url)  
Fig. 4 Storage time equivalent to peroxide value (POV) below 20 as function of the difference between the storage temperature and glass transition temperature ($T-T_g$) of spray dried sea buckthorn seed oil powders prepared of MD–GA and HiCap. The grey and black colours of the symbols indicate the storage temperature of the powders 50 and 20°C, respectively [6].
conductance based on impedance spectroscopy under variable relative humidity and at different temperatures. The activation energy for KCl diffusion was calculated using Arrhenius plot \((\log D \text{ vs } 1/T)\), and a linear decrease was obtained with increasing water content. No kink was present at \(T_g\), indicating decoupling of KCl diffusion and viscosity (Fig. 5).

The observation that water indeed was mobile in glassy carbohydrates may indicate that also gases are diffusing with significant rates in glassy systems. The preservation efficiency of glassy biomaterials was rather recently said to be dependent on water mobility which in turn was claimed to depend on local, segmental molecular motion [10]. By stiffening the segmental motions of the matrix carbohydrates preservation efficiency can be improved. Thus, both translational and local motions of the matrix molecules need to be frozen to achieve the best performance. Furthermore, recent investigations indicate that the \(T_g\) of the carbohydrate matrix is not the only important property in a glassy state, but also the matrix density affects the stability [4]. Ubbink suggested that in order to optimise the matrix carbohydrate composition for the best preservation performance, a compromise between \(T_g\) and density needs to be made. The increase in density could be very likely linked with the decrease of local motion in a glassy state.

3.2 Better performance of spray-dried powder via interfacial engineering

In order to be able to react with the oil in a spray-dried powder particle, oxygen has to diffuse not only across the matrix but also across the interface between the oil droplet and the matrix. It has also been suggested that properties of the interface such as compactness, thickness and rheology could be important factors influencing the rate of mass transfer across the dispersed phase of liquid emulsions [11]. If the interface is important in liquid emulsions it may also affect the performance of dried emulsions. Because the interface around an oil droplet of dried powders is difficult to investigate, we decided to try to study emulsion interfaces and specifically oxygen diffusion across such interfaces (Fig. 6). We applied a

![Fig. 5](image-url) The activation energy for KCL diffusion in the amylose film (□), and for water diffusion in starch (■) taken from Fish [9] as the function of water content. The glass transition temperature of the amylose film is shown (\(T_g\)).

![Fig. 6](image-url) Experimental (separate points, ■, ▲, □, △, ○) and theoretical (lines and dotted lines, model 1) curves for emulsions stabilized with Na-caseinate (■), whey protein (□), △, pectin (△), and combinations of Na-caseinate and pectin (○, ▲, △). The schematic picture on the right describes the oil droplet covered with the emulsifier interface, and shows the Fick’s second law equation for a spherical particle.
method published very recently [1] for liquid emulsions with the aim to generate useful information which could later be applied for dried emulsions. A fluorescent dye, which forms a reversible complex with oxygen, was added to the oil phase, emulsion prepared, oxygen removed from the emulsion and the fluorescent intensity was immediately measured as a function of time with a plate reader. Dye showed maximum intensity with no oxygen and decreased in intensity with increasing oxygen concentration.

Emulsions stabilized with proteins and carbohydrates were prepared and analyzed with the plate reader. The obtained intensity vs. time curves were fitted with the two equations described in Materials and Methods to calculate the diffusion coefficient. As an example of fitting the experimental data with the theoretical model 1 is shown in Fig. 6 and demonstrates the performance of emulsions prepared from milk proteins and pectin. The experimental data did not fit well with the model 1, which was the case with the model 2 as well. The calculated diffusion coefficients for oxygen were in the range of $10^{-14}$–$10^{-15}$ cm$^2$/s, not much depending on the model used. These values were about ten times smaller than what Tikekar et al [1] reported for very similar interfaces.

The diffusion coefficient of oxygen in a good oxygen barrier film of ethylene vinyl alcohol (EVOH) was reported to be equal to $10^{-9}$ cm$^2$/s at a relative humidity of 90% [12]. This indicates that the values obtained above for the emulsion interfaces cannot be correct. This may be due to the experimental set-up applied. The initial conditions assumed in the models, i.e. the saturation of oxygen in the continuous phase and zero oxygen concentration in the oil phase for each oil droplets in the emulsions may not be fulfilled.

We also applied scanning electrochemical microscopy to determine the oxygen diffusion coefficient across air–water interfaces made of milk proteins and obtained much larger and more realistic values than the above presented diffusion coefficients [13]. Hence, we suggest that the electrochemical method is a more potential technique than Tikekar’s method which, however, has the advantage that it can be applied also for oil–water interfaces.

Finally, the trials to measure the diffusion coefficient of oxygen across such thin interfaces found in protein stabilized emulsions, with thicknesses typically from 10 to 100 nm, raised a question whether this route is worth to investigate further? The time needed to cross an interface can be evaluated using Einstein’s equation, thickness $\sim (Dt)^{1/2}$. For an interface with the thickness of 10 nm, the time is 0.005 seconds, 8 minutes and 580 days with the diffusion coefficients of $10^{-10}$, $10^{-15}$ and $10^{-20}$ cm$^2$/s, respectively. Thus, extremely low values for diffusion coefficient are necessary to achieve a significantly lower rate of oxygen transport. This simple calculation indicates that the manipulating an emulsion interface is probably not the most fruitful route for an improved oxidative stability of oils dispersed in powders. Furthermore, this indicates that oxygen transfer may not be the explaining factor for improved oxidative stability of an emulsion with modified interfacial layer as demonstrated in our recent work [14].

One additional aspect needs to be considered in the diffusion process. As oxygen diffuses across a matrix or an interface, it necessarily involves that oxygen is to some extent soluble in the material. If oxygen is not soluble no transfer can take place, which means that solubility is a crucially important factor. Thus, not only the physical properties of the matrix or the interface materials such as glassy state or density are important but also the chemistry matters, affecting the overall performance.

4. Conclusions

In order to develop good carriers, such as spray–dried emulsions for protection of sensitive lipids against oxidation, the most important factors are the oxygen equilibrium and dynamics in the matrix. Carrier matrix composition should be optimized with regard to the glass transition temperature and density, and if possible, further modifications to decrease the segmental molecular motion in the glassy state could be beneficial. Since the interface surrounding the dispersed oil phase is normally very thin, the interfacial engineering towards improved protection against oxidation may not be the most fruitful route for a better overall performance of the powder particles. However, in liquid systems, interface modification may decrease the contact between the phases and thus reduce catalytic activity of the aqueous phase. As Molinero and Goddard have assumed, the major challenge for a better preservation property of glassy carbohydrates is to slow down water mobility, which strongly indicates that a better protection will be achieved by developing less water sensitive matrices.

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References

1) R. Tikekar, A. Johnson, N. Nitin; Real-time measurement of oxygen transport across an oil–water emulsions interface. J. Food Eng., 103, 14–20 (2011).
2) P. Myllärinen, R. Partanen, J. Seppälä, P. Forssell; Effect of glycerol on behavior of amylose and amylopectin films. Carbohydrate Polym., 50, 355–361 (2002).
3) R. Partanen, H. Yoshii, H. Kallio, B. Yan, P. Forssell; Encapsulation of sea buckthorn kernel oil in modified starches. JAOS, 79(3), 219–223 (2002).
4) J. B. Ubbink; Chapter 9; Structural Advances in the Understanding of Carbohydrate Glasses, in: S. Kasapis, I. T. Norton, J.B. Ubbink eds., Modern Biopolymer Science, Academic Press, Elsevier, 2009, pp. 277–293.
5) R. Partanen, P. Hakala, O. Sjövall, H. Kallio, P. Forssell; Effect of relative humidity on the oxidative stability of microencapsulated sea buckthorn seed oil. J. Food Sci.; 70(1), E37–E43 (2005).
6) R. Partanen; Mobility and oxidative stability in plasticized food matrices, Dissertation, Helsinki University of Technology, 2008.
7) R. Partanen, J. Raula, R. Seppänen, J. Buchert, E. Kauppinen, P. Forssell; The effect of relative humidity on oxidation of flaxseed seed oil in spray dried whey protein emulsions. J. Agric. Food Chem., 56, 5717–5722 (2008).
8) R. H. Tromp, R. Parker, S. G. Ring; Water diffusion in glasses of carbohydrates. Carbohydrate Res., 303, 199–205 (1997).
9) B. P. Fish; Diffusion and equilibrium properties of water in starch, Ph.D. thesis, University of Cambridge, 1957.
10) V. Molinero, W. A. Goddard III; Microscopic Mechanism of Water Diffusion in Glucose Glasses. Phys. Rev. Letters, 95, 045701–1 (2005).
11) D. Langevin; Influence of interfacial rheology on foam and emulsion properties. Adv. Colloid Sci. and Int. Sci., 88, 209–222 (2000).
12) C. L. de Dicastillo, C. Nerín, P. Alfaro, R. Catala, R. Gavara, P. Hernandez–Munoz; Development of new antioxidant active packaging films based on ethylene vinyl alcohol copolymer (EVOH) and green tea extract. J. Agric. Food Chem., 59, 7832–7840 (2011).
13) O. Toikkanen, M. Lähteenmäki, T. Moisio, P. Forssell, R. Partanen, L. Murtojoki; Study of oxygen transfer across milk proteins at air–water interface with scanning electrochemical microscopy. J. Agric. Food Chem., 62, 2284–2288 (2014).
14) H. Ma, P. Forssell, P. Kylli, A-M. Lampi, J. Buchert, H. Boer and R. Partanen; Transglutaminase catalyzed cross-linking of sodium caseinate improves oxidative stability of flaxseed oil emulsion. J. Agric. Food Chem., 60, 6223–6229 (2012).