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Impact of Physicochemical Characteristics on the Oxidative Stability of Fish Oil Microencapsulated by Spray-Drying

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The aim of the present research was to identify principal parameters determining the oxidative stability of microencapsulated fish oil. Microcapsules were prepared by spray-drying using different types of *n*-octenylsuccinate-derivatized starch, gum Arabic, sugar beet pectin, sodium caseinate, and/or glucose syrup. Two principal components to classify the different microcapsules accounting for up to 79% of the variance were identified. The principal components were determined by physicochemical parameters reflecting the emulsifying ability of the encapsulant and the drying behavior of the parent emulsion. Microcapsules, which were identified by principal component analysis to be significantly different, exhibited a low stability upon storage, showing that the principal components and, thus, the underlying physicochemical parameters analyzed in the present study are correlated with core material stability.

KEYWORDS: Wall material; matrix; polyunsaturated fatty acids; particle properties; lipid oxidation

INTRODUCTION

Within the range of functional foods, foods supplemented with omega-3 fatty acids, and especially with long-chain polyunsaturated fatty acids (LCPUFAs) docosahexanoic acid and eicosapentanoic acid from marine sources, have attracted much attention in the past decade. Nutritionists consider the fortification of foods with LCPUFAs as highly desirable and safe, and epidemiological studies suggest that a diet high in LCPUFAs may exert a variety of beneficial health effects, e.g., prevention from atherosclerosis and protection against arrhythmias (*1*). Intake of LCPUFAs is generally too low in the diet of Western developed countries, and it is recommended to increase the intake of these acids by supplementation of selected foods (*2*).

Generally, LCPUFAs are suitable for incorporation into foods, but due to their high degree of unsaturation it is difficult to protect them from lipid oxidation. Microencapsulation techniques offer the possibility for the protection and controlled release of lipophilic functional food ingredients and can be used for supplementation of foods with LCPUFAs. The wall materials for microencapsulation can be classified into gums (gum Arabic, locust bean gum, agar agar), lipids (wax, palm fat), proteins (gelatin, milk proteins, soy protein), polysaccharides (starch, xanthan, pullulan, guar gum, alginate), and mono-, di-, and oligosaccharides (hydrolyzed starch, lactose) as well as cellulose and its derivatives (carboxymethylcellulose, methylcellulose). However, not all of these wall materials are suitable for microencapsulation by spray-drying, and Desai and Park (*3*) recently highlighted that one limitation of the spray-drying technique is the limited number of wall materials available. The authors (*3*) also emphasized that the development of alternative and inexpensive polymers that may be considered natural, like gum Arabic, and that could encapsulate, e.g., flavors with the same efficiency than gum Arabic, is an area of research of increasing interest.

A number of studies on the microencapsulation properties of different wall materials for lipophilic food ingredients are readily available. The wall materials used include gum Arabic (*4–6*), gum Arabic and/or *n*-octenylsuccinate-derivatized (nOSA)-starch (*7–12*), nOSA-starch (*13, 14*), mesquite gum (*15*), sugar beet pectin (*16*), or milk proteins (*17–23*). Unfortunately, several of these studies lack a proper characterization of the process and the microcapsule characteristics, use insensitive methodology for determination of lipid oxidation, or result in microcapsules with an insufficient degree of microencapsulation efficiency. The available studies on the influence of composition and process conditions on the physicochemical properties and stability of microencapsulated lipophilic food

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Table 1. Composition of Different Microcapsules (%)

	type of wall material	%
1	nOSA-starch type 1	27.0
2	nOSA-starch type 1/glucose syrup	4.5/22.5
3	nOSA-starch type 2	27.0
4	nOSA-starch type 2/glucose syrup	4.5/22.5
5	nOSA-starch/gum Arabic/glucose syrup	6.7/13.5/6.7
6	gum Arabic/glucose syrup	18.0/9.0
7	sugar beet pectin/glucose syrup	1.0/26.0
8	caseinate/glucose syrup	5.4/21.6
9	caseinate/glucose syrup	1.3/25.6

ingredients have recently been reviewed. All available studies are empirical, and no general conclusions for the prediction of the suitability of new encapsulant systems can be derived. Furthermore, due to differences in the core material, the encapsulant, process parameters, and methodology, a combined analysis of the available studies is not possible. Baik et al. (*25*) also recently pointed out that lipid oxidation in the dry state is far from understood. The aim of the present research was to investigate the contribution of physicochemical characteristics of different microcapsules on their oxidative stability and to identify principal parameters determining the oxidative stability of microencapsulated lipophilic core materials.

MATERIALS AND METHODS

Commercially available refined fish oil stabilized with mixed tocopherols was provided by Denomega Nutritional Oils A/S (Gamle Frederikstad, Norway). The fish oil contained approximately 36% omega-3 fatty acids. The concentration of each of the long-chain polyunsaturated fatty acids eicosapentanoic acid and docosahexanoic acid amounted to 14%. Microcapsules were prepared with an oil load of 40% of the dry matter content. Parent emulsions were prepared at 45% total solids; i.e., the oil load of the parent emulsion was 18%. The different wall material systems of the individual samples are summarized in **Table 1**.

Two different types of nOSA-starch have been chosen and were used either as single wall material or in combination with glucose syrup. nOSA-starch is usually hydrolyzed to reduce the viscosity of the emulsion according to the target application, and the two different starches used in the present study differed in type of enzyme used for hydrolysis and thus in molecular weight distribution. nOSA-starch was provided by Cargill Deutschland GmbH and National Starch & Chemicals. Sugar beet pectin (Genutype Beta) was a kind gift from CP Kelco Germany GmbH and was used at a level of 1% of the total dry matter in the composition according to the recommendation of Drusch (*16*). Caseinate was supplied by Condio GmbH. The combination with glucose syrup in a ratio of 1/4 and 1/19 was chosen on the basis of the results of Hogan et al. (*19*) and data from our laboratory on microencapsulation efficiency depending on the glucose syrup/caseinate ratio (unpublished data). Gum Arabic was purchased from Alfred Wolff (Quick Gum type 8074); another quality especially developed for encapsulation purposes was provided by Colloides Naturel International. A combination of nOSA-starch with gum Arabic to increase stability of microencapsulated core material was suggested by Kanakdande et al. (*10*) and Krishnan et al. (*7*, *8*). The composition of the microcapsules with gum Arabic as emulsifying wall material was chosen according to the results of McNamee et al. (*5*) and the manufacturers' recommendation for its use for encapsulation of flavorings.

Generally, after dissolution of the water-soluble compounds a coarse emulsion was prepared by subsequent homogenization at 50 bar in a high-pressure homogenizer (Panda 2K; Niro Soavi Deutschland, Lübeck, Germany) followed by a final two-step homogenization. Spray-drying was carried out at 180/70 °C on a laboratory scale spray-dryer (1–7 kg/h water evaporative capacity; Mobile Minor, Niro A/S, Denmark) equipped with a rotating disk for atomization. For monitoring lipid oxidation, samples were stored in duplicate protected from light in open glass bottles at 20 °C in a desiccator over a saturated solution of magnesium chloride resulting in a relative humidity of 33%.

Viscosity of the emulsions was determined using a rotational viscometer (Haake Viscotester 7L; Thermo Electron Corp., Dreiech, Germany). All oil droplet size and particle size analyses were determined using a laser diffraction sensor (Helos; Sympatec GmbH, Clausthal-Zellerfeld, Germany) equipped with a cuvette. Results are reported as 50th and 90th percentile of the size of the particles. Oil droplet size of the emulsions was determined after dilution of the sample with water; for analysis of the oil droplet size of the microcapsules, an aliquot of powder was reconstituted in water. Particle size analysis of the microcapsules was performed after dispersing an aliquot of the powder in an inert oil (Miglyol 812; Sasol Germany, Hamburg, Germany). All analyses were performed with four replicates.

Particle surface area and apparent density were determined with the Brunauer–Emmett–Teller (BET) method (*26*) using a Nova 2200 high-speed gas sorption analyzer (Quantachrome GmbH, Odelzhausen, Germany). As the most widely used gas for surface area determinations nitrogen (at 77 K) served as adsorbate, considering a multilayer sorption. The determination of pore volume and maximum pore size is based on the Kelvin equation and was accomplished with the same equipment after the method proposed by Barrett et al. (*27*). The porosity analysis ranges from micropores (<20 nm) that are still permeated by nitrogen molecules to macropores (>500 nm) that are still filled due to capillary condensation (see maximum pore size). True density of the powder particles was determined using a Pycnomatic helium pycnometer (Thermo Electron Corp., Dreiech, Germany).

Water activity after spray-drying was measured using a TH500 AW Sprint (Novasina, Pfäffikon, Switzerland); moisture content was analyzed using an infrared moisture analyzer, MA30 (Sartorius, Göttingen, Germany). The amount of extractable oil was determined according to the method described by Westergaard (*28*). A CamScan 44 REM/EDX scanning electron microscope (CamScan USA Inc., Cranberry Township, PA) was used to view and describe the characteristics of the microcapsules.

Oil was extracted from the microcapsules after reconstitution with water using a blend of ethanol, hexane, and ethyl acetate as described by Satué-Gracia (*29*). Lipid oxidation was monitored through the analysis of hydroperoxide content and volatile secondary lipid oxidation products by static headspace gas chromatography with mass selective detection. Hydroperoxide concentration was determined using the IDF standard method 74A:1991 for the determination of the peroxide value in anhydrous milk fat with slight modifications (*30*). 2-Propanol was used as solvent in the test protocol. After addition of the iron(II) chloride and the ammonium thiocyanate solution, samples were incubated in a water bath at 60 °C for 30 min. The extinction was measured at a wavelength of 485 nm. All analyses of hydroperoxides were performed with three replicates.

For the determination of propanal via static headspace gas chromatography, 1 g of powder was weighed in 20 mL crimp-sealed glass vials and was redissolved by adding 2 mL of EDTA solution (0.5%). Samples were equilibrated at 70 °C for 15 min. An aliquot of the headspace (1 mL) was injected into an Agilent 6890 gas chromatograph equipped with a HP-Innowax column (60 m × 0.32 mm × 0.5 μm) and an Agilent 5975 inert mass selective detector. The injector was operated in the split mode (5.3:1). Injector and detector temperatures were set at 270 and 250 °C, respectively. Initially, the oven temperature was set at 50 °C and was held for 1.5 min. The temperature was increased to 240 °C at a rate of 20 °C min^{-1}, where it was held for 3 min. The mass spectrometer was operated in the electron ionization mode (70 eV), and data were acquired in the full-scan mode for the range m/z 20–200. The temperature of the ion source and the detector was 150 and 230 °C, respectively. Propanal was identified using the retention time of an external standard and by reference to the NIST library (NIST/EPA/NIH Mass Spectral Library, Version 2.0d, National Institute of Standards and Technology, Manchester, U.K.). The target ion was 58; qualifiers were 29, 28, and 27. Quantification of propanal was done after calibration with known amounts of propanal standard.

Data on the physicochemical characterization of the microcapsules were analyzed using the principal component analysis (PCA). PCA identifies the underlying factors that influence a system. A new set of

Table 2. Physicochemical Properties of the Parent Emulsion and Spray-Dried Microcapsules with nOSA-Starch or a Mixture of nOSA-Starch and Glucose Syrup

parameter	unit	nOSA type 1	nOSA type 1, glucose syrup	nOSA type 2	nOSA type 2, glucose syrup
viscosity	mPa s	1824	52	193	32
oil droplet size, parent emulsion	50th percentile, μm	1.24 ± 0.00	1.30 ± 0.01	0.90 ± 0.00	1.21 ± 0.01
oil droplet size, parent emulsion	90th percentile, μm	2.49 ± 0.02	2.58 ± 0.04	1.88 ± 0.01	2.46 ± 0.14
dry matter	%	96.9	97.5	98.0	98.0
a_w value		0.129	0.118	0.138	0.101
extractable oil	%	4.1 ± 0.3	5.4 ± 0.4	2.0 ± 0.1	5.4 ± 0.1
oil droplet size, redissolved emulsion	50th percentile, μm	1.15 ± 0.04	1.26 ± 0.00	0.90 ± 0.01	1.18 ± 0.01
oil droplet size, redissolved emulsion	90th percentile, μm	2.48 ± 0.19	2.51 ± 0.01	1.91 ± 0.06	2.59 ± 0.14
particle size	50th percentile, μm	30.7 ± 1.6	21.4 ± 1.0	20.9 ± 0.8	19.9 ± 0.8
particle size	90th percentile, μm	51.8 ± 3.0	44.4 ± 1.4	45.0 ± 1.3	43.2 ± 2.1
true density	g/mL	1.118 ± 0.003	1.199 ± 0.002	1.179 ± 0.003	1.203 ± 0.002
apparent density	g/mL	1.008 ± 0.010	1.142 ± 0.013	1.099 ± 0.015	1.094 ± 0.007
particle surface	m^2/g	0.877 ± 0.048	0.651 ± 0.031	0.884 ± 0.001	0.794 ± 0.040
pore volume	mL of pore/mL of sample	1.23E-03	1.52E-03	1.46E-03	1.35E-03
maximum pore size	nm	133	867	142	98

variables, the principal components, is created from the original data matrix. In the present study, a data matrix with 9 rows (samples with different encapsulants) and 15 columns (variables, physicochemical data) was created. Principal component analysis was performed by using the statistical package SCAN (Minitab Inc., PA, 1995). Results are presented as two scatter plots, the score plot and loading plot, in which the samples (score plot) or the data on the physicochemical characterization (loading plot) are projected onto the principal components.

RESULTS AND DISCUSSION

Table 2 summarizes the data on the physicochemical characterization of the parent emulsions and microcapsules prepared using either nOSA-starch or nOSA-starch in combination with glucose syrup. With 1824 mPa s, the parent emulsion prepared with nOSA-starch type 1 showed a far higher viscosity than the other three parent emulsions. The 50th percentile of the oil droplet size of all parent emulsions was below 1.5 μm, indicating sufficient stability of the parent emulsions for spray-drying.

All spray-dried microcapsules had a dry matter content of at least 96.9% and an a_w value of 0.138 and can thus be considered to possess long-term stability against microbiological spoilage. Extractable oil in the microcapsules prepared with nOSA-starch amounted to 2.0% and 4.1% of the total oil content, while the amount of extractable oil was higher in the microcapsules prepared with a mixture of nOSA-starch and glucose syrup (5.4% of the total oil content). The lowest proportion of extractable oil occurred in the sample with the smallest oil droplet size. Other studies also reported a positive correlation between oil droplet size and extractable core material for individual encapsulation systems (*31*, *32*). However, when comparing all of the different encapsulation systems in the present study, no correlation was found between droplet size in the parent emulsion and extractable oil in the dried powder. Extractable oil is partially located at the particle surface and is directly associated to the flowability and wetting properties of the powder as well as the stability toward oxidation (*33*). In the nOSA-starch-based microcapsules, oil droplet size of the reconstituted emulsion was unchanged compared to the parent emulsion, indicating that the emulsion was stable during atomization and subsequent drying. The particle size of the microcapsules prepared from the parent emulsion with a high viscosity (nOSA type 1, particle size 30.7 μm, **Table 2**) was higher than the particle size of the other three microcapsules. **Figure 1A** shows that this increase in particle size was not attributed to "ballooning" of the particles as it was, e.g., reported for microcapsules prepared at 210/90 °C when using nOSA-starch and glucose syrup (*14*). Ballooning is a phenomenon, which is caused by fast fixing of the particle structure in the early stage of drying with subsequent steam formation in the interior of the particle and inflation of the particle (*34*). For microcapsules prepared using nOSA-starch type 1 ballooning has been described to occur at a drying temperature of 210/90 °C (*14*). The increase in particle size in the present study was attributed to the generation of larger droplets during atomization due to the higher viscosity of the parent emulsion. Microcapsules prepared with nOSA-starch type 1 also showed a lower apparent and true density of 1.118 and 1.008 g/mL, respectively, compared to the other three microcapsules, indicating a trapping of small air bubbles inside the highly viscous emulsion leading to a higher porosity inside the particle. Pore volume and maximum pore size were in a similar range for all samples, apart from the maximum pore size for the sample prepared from nOSA-starch type 1 and glucose syrup.

The physicochemical characteristics of the gum Arabic-, sugar beet pectin-, and caseinate-based parent emulsions and microcapsules are summarized in **Table 3**. With 432−584 mPa s, viscosities of the parent emulsions containing gum Arabic or sugar beet pectin were markedly higher than the viscosity of the caseinate-based parent emulsions (maximum 134 mPa s). Due to the increased viscosity and the molecular weight and size of the emulsifying compounds, the 50th percent of the oil droplet size in the parent emulsions was higher compared to the oil droplet size of the nOSA-starch-containing emulsions and also the caseinate-containing emulsions. A higher oil droplet size for emulsions prepared from gum Arabic compared to emulsions prepared from nOSA-starch has also been reported by Reineccius (*35*).

All of the microcapsules prepared from gum Arabic, sugar beet pectin, or caseinate were of a similar size of approximately 20−24 μm. Apparent and true densities were also in a similar range with the caseinate-based microcapsules showing slightly lower values for both parameters. Extractable oil ranged from 8.4% of the total oil in the gum Arabic/n-OSA-starch type 1 composition to 25.9% of the total oil in the sugar beet pectin-based microcapsules and was thus higher than in the nOSA-starch-based microcapsules. On the basis of previously published data on the microencapsulation efficiency in relation to oil load (*16*), we already pointed out that the use of sugar beet pectin may be limited by the maximum oil load of the capsules. In the sugar beet pectin-based microcapsules an increase in oil

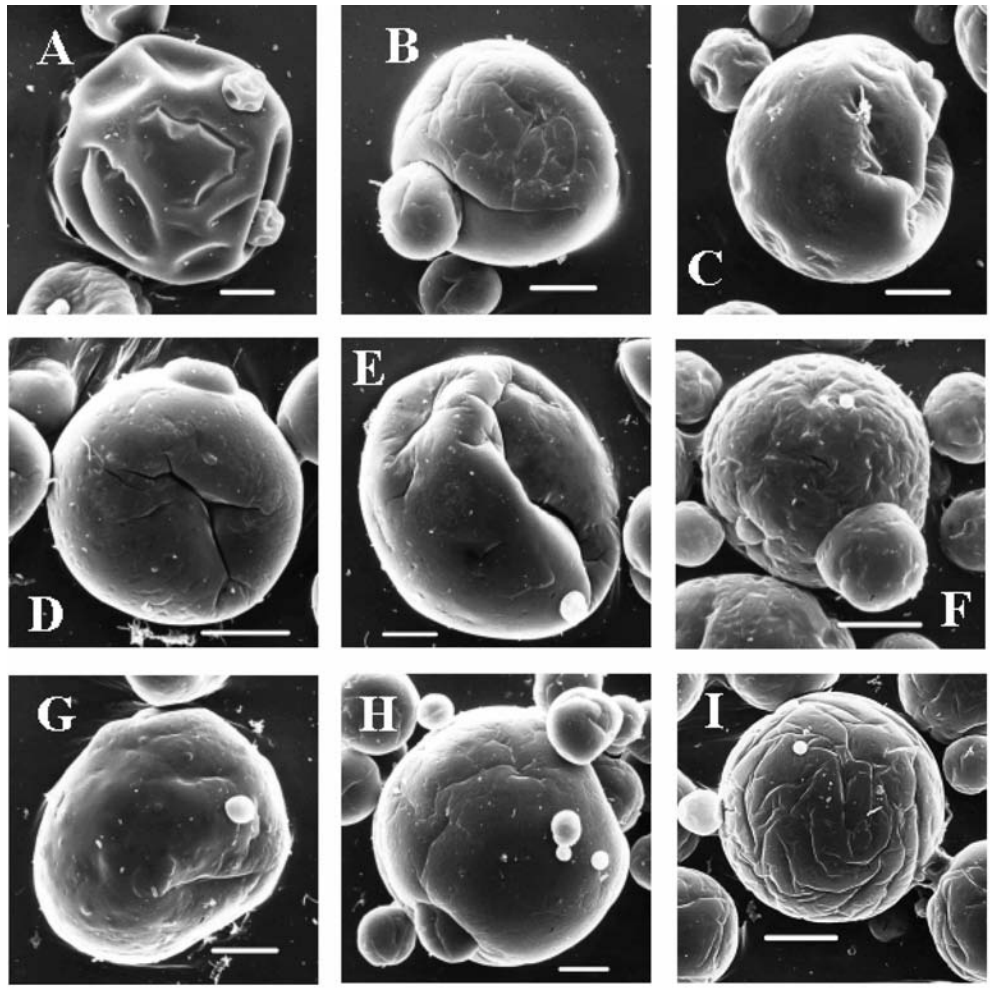

Figure 1. Scanning electron micrographs of microencapsulated fish oil spray-dried at 180/70 °C (**A**, nOSA-starch type 1; **B**, nOSA-starch type 1/glucose syrup; **C**, nOSA-starch type 2; **D**, nOSA-starch type 2/glucose syrup; **E**, nOSA-starch type 1/gum Arabic; **F**, gum Arabic; **G**, sugar beet pectin; **H**, caseinate/glucose syrup, 1/4; **I**, caseinate/glucose syrup, 1/19). White bar = 10 μm.

droplet size after spray-drying compared to the parent emulsion was observed for both the 50th and the 90th percentile of the oil droplet size, indicating coalescence of the extractable oil after reconstitution of the microcapsules. A slight increase in the oil droplet size from 1.28 to 1.57 μm for the 50th percentile also occurred in one of the caseinate-based microcapsules (caseinate/glucose syrup, 1/4). A significant decrease in oil droplet size was observed for the gum Arabic-containing microcapsules. According to Soottitantawat et al. (*31*) large emulsion droplets can be sheared into smaller droplets because of the large velocity gradient and the turbulence when using rotary atomization and may explain the present results.

Pore volume was significantly lower in the sugar beet pectin-based microcapsules. Taking into consideration the high amount of extractable oil and the scanning electron micrograph (**Figure 1G**), it seems likely that extractable oil contributed to a certain degree to the low pore volume by covering pores in the particle surface. Another explanation for the low pore volume is the high proportion of low molecular weight carbohydrates in the encapsulant allowing a dense packaging within the wall. In the present study, the caseinate-based microcapsules also showed a low pore volume, which can be explained by the formation of a thick film at the powder surface upon spray-drying as was reported for caseinate and different low molecular weight carbohydrates by Elofsson and Millqvist-Fureby (*36*).

To provide partial visualization of the data set in a reduced dimension, thus summarizing the data and allowing the identification of the important differences in the physicochemical characteristics of the particles, principal component analysis was performed on the autoscaled data. The first two principal components represent 68% of the total variance. Examining the score plot (**Figure 2**) in the area defined by the first two principal components, a clear separation of the samples into three groups was found. The first principal component was able to discriminate the gum Arabic- and the sugar beet pectin-containing microcapsules from the other compositions. The loading plot in **Figure 2** shows the relationship between the physicochemical characteristics and their contribution to the macrovariables. The oil droplet size, the extractable oil, and the particle surface were dominant in the first principal component. High molecular weight emulsifying constituents are less mobile in solution compared to low molecular weight emulsifiers. The stabilization of the newly formed interface during homogenization is comparably slow, and oil droplet coalescence may occur prior to complete coverage of the newly formed interface with emulsifier reflected by an increase of the oil droplet size. Thus, principal component 1 reflects the molecular weight and the emulsifying ability of the encapsulant during homogenization. An increase in oil droplet size has been associated with a decrease in microencapsulation efficiency (*31*, *38*) and explains why both oil droplet size and extractable oil determine the distinction of the microcapsules by principal component 1.

The second principal component was able to discriminate the nOSA-starch type 1-based microcapsules from all other com-

Table 3. Physicochemical Properties of the Parent Emulsion and Spray-Dried Microcapsules with Gum Arabic, a Combination of Gum Arabic and nOSA-Starch, Sugar Beet Pectin, or Caseinate

parameter	unit	gum Arabic nOSA type 1, glucose syrup	gum Arabic, glucose syrup	sugar beet pectin, glucose syrup	caseinate, glucose syrup (1/4)	caseinate, glucose syrup (1/19)
viscosity	mPa s	432	584	561	134	32
oil droplet size, parent emulsion	50th percentile, μm	1.56 ± 0.00	6.21 ± 0.29	1.57 ± 0.01	1.28 ± 0.01	1.26 ± 0.01
oil droplet size, parent emulsion	90th percentile, μm	3.49 ± 0.01	17.3 ± 1.89	2.99 ± 0.01	2.37 ± 0.02	2.26 ± 0.01
dry matter	%	96.9	97.7	96.3	97.6	98.0
a_w value		0.130	0.125	0.137	0.111	0.115
extractable oil	%	8.4 ± 0.3	16.3 ± 0.9	25.9 ± 0.5	13.0 ± 0.1	13.0 ± 0.4
oil droplet size, redissolved emulsion	50th percentile, μm	1.54 ± 0.05	2.50 ± 0.08	2.25 ± 0.06	1.57 ± 0.02	1.32 ± 0.01
oil droplet size, redissolved emulsion	90th percentile, μm	3.63 ± 0.14	7.66 ± 0.26	6.21 ± 0.33	4.33 ± 0.17	2.50 ± 0.02
particle size	50th percentile, μm	23.6 ± 1.0	20.4 ± 1.1	24.2 ± 1.5	22.8 ± 1.5	23.3 ± 0.7
particle size	90th percentile, μm	46.9 ± 2.6	50.7 ± 4.7	49.2 ± 4.8	43.7 ± 4.6	49.2 ± 5.7
true density	g/mL	1.196 ± 0.001	1.211 ± 0.001	1.210 ± 0.002	1.142 ± 0.001	1.172 ± 0.001
apparent density	g/mL	1.088 ± 0.007	1.110 ± 0.008	1.107 ± 0.007	1.017 ± 0.033	1.073 ± 0.005
particle surface	m^2/g	0.727 ± 0.155	0.450 ± 0.044	0.596 ± 0.008	0.651 ± 0.038	0.602 ± 0.051
pore volume	mL of pore/mL of sample	1.04E-03	1.02E-03	8.22E-05	9.96E-04	5.45E-04
max pore size	nm	146	124	139	107	135

positions and reflects a unique drying behavior of this highly viscous composition caused by the interdependency of viscosity, particle size, and true and apparent density as described above. By removing variables, which did not contribute to the model, the contribution of the first two principal components was increased to 79.3% of the total variance.

Hydroperoxides were not detectable in the fish oil itself, but in the emulsion after homogenization. In all parent emulsions, the hydroperoxide content was below 0.5 mmol/kg of oil, apart from the gum Arabic-containing parent emulsions. In the emulsion consisting of gum Arabic or a combination of gum Arabic and nOSA-starch the hydroperoxide content amounted to 6.1 and 2.2 mmol/kg of oil, respectively. These results clearly show that lipid oxidation may already be induced during homogenization and that the selection of antioxidants to prevent microencapsulated oils must aim at the protection of both oil-in-water emulsions and dry capsules.

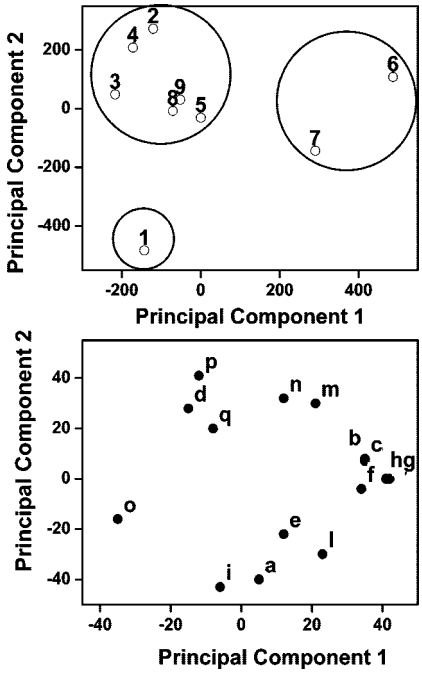

Figure 2. Score plot and loading plot for the principal component analysis of fish oil microencapsulated into different matrices. (Top) Score plot: sample coding according to **Table 1**. (Bottom) Loading plot: a, viscosity; b, 50th percentile of the oil droplet size; c, 90th percentile of the oil droplet size; d, dry matter; e, a_w value; f, extractable oil; g, 50th percentile of the oil droplet size of the reconstituted emulsion; h, 90th percentile of the oil droplet size of the reconstituted emulsion; i, 50th percentile of the particle size; l, 90th percentile of the particle size; m, true density; n, apparent density; o, particle surface; p, pore volume; q, maximum pore size.

Upon storage at 20 °C and 33% relative humidity, the highest and fastest increase for the nOSA-starch-containing microcapsules in both hydroperoxide content and propanal content was observed in the microcapsules prepared with nOSA-starch type 1 (**Figure 3**). Since particle surface, pore volume, and maximum pore size (the determinants of principal component 1) are in a similar range for the microcapsules prepared from nOSA-starch type 1 and type 2, these parameters cannot explain the difference in oxidative stability. Air inclusion in the particle possibly contributes to the decreased stability of microcapsules prepared from nOSA-starch type 1. A more important factor may be the composition and the density of the oil–water interface. The two types of nOSA-starch significantly differ in the molecular weight profile. nOSA-starch type 2 showed a high proportion of disaccharides (data not shown), which is in close agreement with the product characteristics as described in U.S. Patent 5.185.176. In contrast, nOSA-starch type 1 showed a very heterogeneous pattern in the molecular mass distribution with distinct peaks at 6922, 16552, and 1064 Da. The low molecular weight of nOSA-starch type 2 allows a more dense packaging of the molecules at the oil–water interface and thus provides a better protection of the oil. The importance of the oil–water interface for the stability of fish oil emulsions and microencapsulated fish oil against lipid oxidation by limiting diffusion of trace metals and oxygen has recently been highlighted by different authors (*39*, *40*).

The highest stability within the four samples was achieved when using a mixture of nOSA-starch and glucose syrup with no difference either in hydroperoxide content or in propanal content. Reineccius (*35*) emphasized that glucose syrup with a high dextrose equivalent value (DE) is less permeable to oxygen and offers better protection to encapsulated flavors compared to carbohydrates with a low DE value. Furthermore, it has been shown that the addition of mono- and disaccharides to maltodextrins reduces pore size in the maltodextrin network and limits oxygen diffusion, emphasizing that not only the dextrose equivalent of a maltodextrin or glucose syrup but also its molecular weight distribution determines the stability of the encapsulated core material (*41*). Generally in the present study,

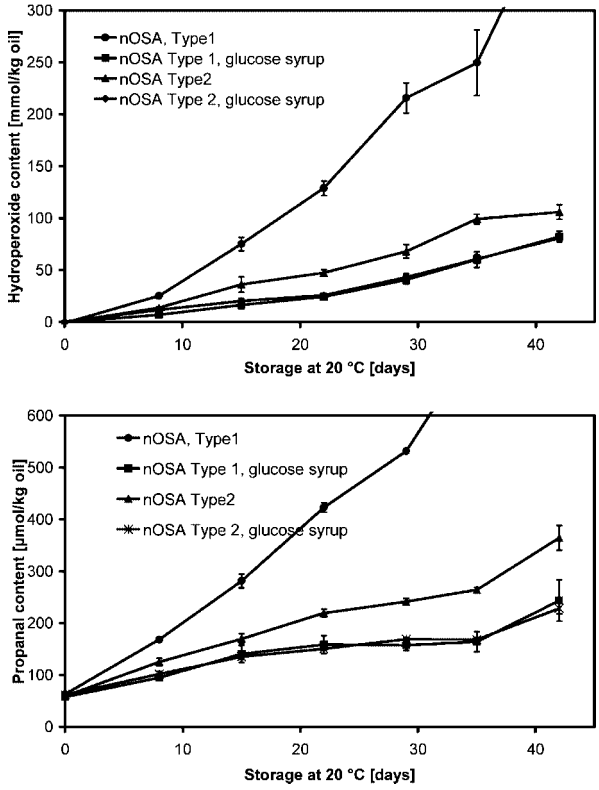

Figure 3. Development of hydroperoxide and propanal content of fish oil microencapsulated with nOSA-starch or a mixture nOSA-starch and glucose syrup during storage at 20 °C and 33% relative humidity.

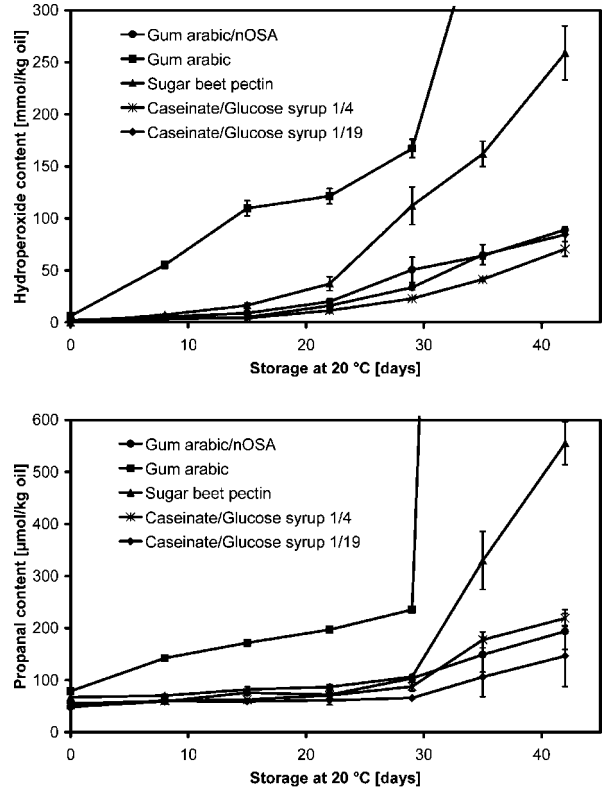

Figure 4. Development of hydroperoxide and propanal content of fish oil microencapsulated with a gum Arabic, a combination of gum Arabic and nOSA-starch, sugar beet pectin, or caseinate during storage at 20 °C and 33% relative humidity.

hydroperoxide content and propanal content showed a very high correlation within the nOSA-starch-based microcapsules (r = 0.989).

In the gum Arabic-, sugar beet pectin-, and caseinate-based microcapsules, the correlation between hydroperoxide content and propanal content was lower (r = 0.799). The lowest stability was observed for the fish oil encapsulated into gum Arabic and glucose syrup (**Figure 4**). According to Righetto and Netto (*42*) the glass transition temperature for gum Arabic at a relative humidity of 33% is 62 °C and the glass transition temperature of glucose syrup (DE36) at 33% relative humidity is 31 °C. Therefore, on the basis of the Gordon−Taylor equation, collapse of the matrix and release of the core material with subsequent oxidation can be excluded since the storage temperature was well below the glass transition temperature of the mixture. The low stability can partially be attributed to the extractable oil, which amounted to 16.3%. Extractable oil is located on the surface of the particle and may oxidize rapidly during storage (*25, 43*). However, the fraction of extractable oil was 25.9% in the sugar beet pectin-based microcapsules, and this sample was more stable than the gum Arabic-based composition. Also, the tremendous improvement in oxidative stability, when using a combination of nOSA-starch and gum Arabic, cannot be explained exclusively by the fraction of extractable oil, which still amounted to 8.4%. Upon reconstitution of the microcapsules no creaming and, apart from the sugar beet pectin-containing microcapsules, no increase in oil droplet size distribution was observed (**Table 3**). This indicates that the extractable oil is not necessarily the result of oil droplet coalescence during spray-drying. The extractable oil fraction obviously also includes microencapsulated oil from the outer layer of the particles. These discrete oil droplets are still surrounded by an interfacial layer that influences the oxidative stability of the extractable oil. We therefore propose to call this fraction "solvent-extractable oil" rather than "nonencapsulated oil" as it is frequently referred to in the literature.

In both gum Arabic and sugar beet pectin low amounts of divalent cations, namely, copper and iron, were detected (data not shown). When combining nOSA-starch and gum Arabic, the metal-chelating activity of excess succinic acid may have contributed to the increase in stability. It is well established that divalent cations catalyze the autoxidation of unsaturated fatty acids, and thus the stability of the encapsulated oil in these formulations can be improved by incorporation of a chelating agent like citric acid. Suitable combinations for stabilization of fish oils including chelating agents have recently been published (*44*). The increased stability of the fish oil encapsulated with a combination of gum Arabic and nOSA-starch is possibly also attributed to a modified composition of the oil–water interface. Due to the lower molecular weight of the nOSA-starch compared to gum Arabic, nOSA-starch molecules have superior mobility and thus more rapidly stabilize the new developed oil–water interface after homogenization. The explanation indicates that the principal component 1 determines oxidative stability of microencapsulated core material.

Interfacial phenomena as outlined above explain the oxidative stability of the caseinate-based microcapsules. These samples showed the highest stability, in a similar range of the stability observed for microcapsules prepared from nOSA-starch/glucose syrup. Hydroperoxide content after 42 days of storage at 20 °C and a relative humidity of 33% reached 70 and 84 mmol/kg of oil in the two caseinate-based compositions. Hogan et al. (*20*) reported a peroxide value of approximately 35 mmol/kg of oil for fish oil encapsulated with caseinate and glucose syrup (DE = 38) after 28 days of storage at 23 °C. However, Hogan et al. (*20*) used a iodometric method for the determination of the peroxide value, which gives different results from those obtained

by determination of the hydroperoxide concentration using the thiocyanate method. Data from the thiocyanate method are higher by a factor of 1.5−2 relative to data of the iodometric method (*45*); data from our laboratory showed a conversion factor of approximately 3 between the methods (unpublished data). Therefore, the hydroperoxide content observed in the present study is in good agreement with the data reported by Hogan et al. (*20*). Furthermore, as reviewed by McClements and Decker (*46*), casein forms a metal-chelating interfacial film around oil droplets and may thus have contributed to the increase in oil stability in the present study by the chelation of divalent cations.

In conclusion, microcapsules, which were identified by PCA to be significantly different, exhibited a low stability upon storage, indicating that the principal components are correlated with microcapsule stability. When eliminating these samples from the data set, a preliminary model to predict oxidative stability could be derived by partial least-squares analysis. Thus, by creating a new data set with a larger variation in the physicochemical properties of the spray-dried capsules, a suitable model for the prediction of the stability of microencapsulated oils can be developed. The results from the present study indicate that the interfacial composition and properties of both the oil–water interface in the parent emulsion and the surface composition of the drying droplet play a major role for core material stability and their characterization should be included in future studies in this field.

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