Stability to Oxidation of Spray-Dried Fish Oil Powder Microencapsulated Using Milk Ingredients

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ABSTRACT: Microencapsulation of fish oil was achieved by spray-drying homogenized emulsions of fish oil using 3 different types of casein as emulsifier and lactose as filler. As the degree of aggregation of the casein emulsifier increased, the vacuole volume of the microencapsulated powders decreased. The shelf life of the powders increased as the degree of aggregation of the casein emulsifier increased at the high homogenization conditions. When micellar casein was used as emulsifier, the shelf life also increased as homogenization conditions increased. Free fat but not surface fat was inversely related to shelf life. Since the type of casein used was confounded with the powder vacuole volume, the increased shelf life may have been due to either factor.

Key Words: Microencapsulation, fish oil, oxidation, stability, milk ingredients

Introduction

MICROENCAPSULATED FAT POWDERS ARE ESSENTIALLY DRIED homogenized emulsions of an oil or fat where proteins, modified starches or hydrocolloids are used as emulsifying materials. A non-emulsifying water-soluble material such as a sugar or hydrolyzed starch is also used as filler (Reineccius 1986). The advantage of microencapsulation for fish oil is that the oil can be converted to powder form where shelf life can be extended by avoiding contact between the fish oil and the pro-oxidant materials present, such as atmospheric oxygen. There have been a number of reports on the use of sodium caseinate and whey proteins as encapsulants and lactose as filler (Rosenberg and Lee 1993; Rosenberg and Young 1993; Young and others 1993a, b; Fäldt and others 1993; Keogh and O'Kennedy 1999). However, reports focusing on reducing the rate of oxidation are fewer (Buma 1971a; Granelli and others 1996). Buma (1971a) showed that oxidative stability was not related to free fat in powders and that free fat was related to porosity (Buma, 1971b). Later, Granelli and others (1996) showed that the rate of oxidation was related to surface fat, as measured by electron spectroscopy for chemical analysis (ESCA) of the powder particle surfaces. Moreau and Rosenberg (1998) showed that the porosity of the matrix to gases was affected by the fat core and whey protein encapsulant levels used, but oxidation of anhydrous milk fat did not occur over a 12-mo period in such matrices (Moreau and Rosenberg 1996). Reineccius (1988) stated that the effect of air in powders had not been investigated. In our study, free fat, surface fat and the air content of the powder particles were measured and related to the shelf life as monitored by sensory evaluation.

Materials and Methods

SPRAY-DRIED FISH OIL POWDERS WERE PREPARED FROM EMULsions containing total solids levels of 30.0 to 34.5%. The emulsions were produced using reconstituted sodium caseinate, calcium caseinate or skim milk powder. In addition to the milk protein powder type used, the effects of homogenization pressure × number of passes and 2 packaging methods were evaluated.

Materials

Fish oils were prepared by the Danish Institute for Fisheries Research (Lyngby, Denmark) from sand eel (Ammodytes species) and stored at −18 °C on arrival. Sodium caseinate (88.0% protein) was obtained from DMV International, BA Veghel, Holland (product code EM 6). Calcium caseinate (88.0% protein) was supplied by Dairygold, Mitchelstown, Co. Cork, Ireland. Skim milk powder (38.0% protein, 50% lactose) containing a mixture of micellar casein and whey proteins in a 76:24 ratio was supplied by The Irish Dairy Board, Dublin, Ireland. Lactose was supplied by Glanbia plc., Ballyragget, Co. Kilkenny, Ireland. An antioxidant mixture, coded ALT 1, was added to the fish oil during preparation by the Danish Institute for Fisheries Research. ALT 1 is a mixture of the naturally occurring antioxidants ascorbic acid (8.6%), lecithin (5.2%) and α-tocopherol (86.2%) that was developed and patented by Löliger and Saucy (1989).

Preparation of the emulsions

The aqueous phase was made by adding skim milk powder (SMP, which contained both protein and lactose) or caseinate and lactose separately to deionized water at 50 to 60 °C, preadjusted to give a final solution pH of 7.0 ± 0.1. The aqueous phase was cooled to 20 to 25 °C before fish oil addition and homogenization. A centrifugation or de-aeration step was not used. The ratio of the components used to prepare standard emulsions was 10 fish oil: 10 caseinate: 10 lactose: 70 water. When SMP was used as encapsulant, the emulsion composition was 11.5 fish oil: 23.0 SMP: 65.5 water. In this way, the first 2 of 4 conditions were met: (i) the fat content remained constant at 1/3 of the total solids; (ii) the protein content, at 8.8% w/w, was the same in each emulsion (protein content of caseinate = 88.0%; protein content of SMP = 38%). (iii) The total solids of the emulsion with SMP was therefore unavoidably increased to 34.5% (that is, 11.5 + 23.0%) compared with the standard 30.0% and (iv) the lactose content was 11.5% (lactose content of SMP = 50%) versus the standard 10.0%. The latter 2 minor changes in conditions were ignored. Fish oil, warmed from −18 °C to 4 °C overnight and to 25 °C in a water-bath at 40 °C until the oil became clear, was mixed into

Table 1—Trial numbers, homogenization pressure × passes, vacuole volume or casein type and packaging system used in main experiment

Trial no.	Homogenization conditions [pressure (MPa) × passes]	Process conditions Vacuole volume (mL/100 g)	Casein type	Packaging[1] system
7	50 × 5	22.6	Sodium caseinate	2
7R[2]	50 × 5	22.2	Sodium caseinate	2
10	15 × 1	18.6	Sodium caseinate	2
8	15 × 1	9.4	SMP	2
1	50 × 5	13.0	Calcium caseinate	1
5	30 × 3	22.0	Sodium caseinate	1
4	15 × 1	6.2	SMP	1
9	30 × 3	6.4	SMP	2
9R	30 × 3	6.4	SMP	2
3	30 × 3	15.2	Calcium caseinate	1
6	15 × 1	19.5	Sodium caseinate	1
3R	30 × 3	15.3	Calcium caseinate	1
4R	15 × 1	5.3	SMP	1
2	50 × 5	6.4	SMP	1
12	50 × 5	5.9	SMP	2
2R	50 × 5	8.1	SMP	1
8R	15 × 1	12.8	Calcium caseinate	2
11	50 × 5	12.8	Calcium caseinate	2
1R	50 × 5	17.5	Calcium caseinate	1
10R	15 × 1	16.5	Calcium caseinate	2
5R	30 × 3	22.7	Sodium caseinate	1

[1]Packaging: 1 = vacuum + N_2 packaging; 2 = vacuum-packaging
[2]R = replicate

the aqueous suspension at 20 to 25 °C and gently stirred using a laboratory mixer emulsifier at the lowest speed of 200 rpm (Silverson Mixer Model AXR; Silverson Machines Ltd., Chesham, U.K.). The coarse emulsions were then homogenized at 20 to 25 °C at single-stage pressures ranging from 15 to 50 MPa and 1 to 5 passes in a Gaulin Mini-Lab homogenizer (APV, Silkeborg, Denmark), at a rate of 60 kg/h. The emulsions (10-kg aliquots) were collected in a chilled water-jacketed tank to keep the temperature below 30 °C. Any fish oil remaining in the container was discarded.

Spray-drying

A pilot-scale dryer (F1 Lab dryer; Anhydro, Copenhagen, Denmark) was used with a 2-fluid nozzle. The air inlet temperature was 177° ± 2 °C and the outlet air temperature was 75 °C ± 2 °C. The powders were vacuum-packed, sealed in aluminum bags and temporarily stored at −18 °C before being subjected to storage evaluation.

Vacuum-packaging

The powders were removed from temporary storage at −18 °C and stored at 4 °C in 200-mL aluminum foil bags (i) under vacuum and (ii) under vacuum + N_2 packaging treatments. The vacuum in the bags (Webomatic Packaging Systems, Bochum, Germany) was drawn to an absolute pressure of 4 mbar, (Busch Vac Control, part no. 545-112, Busch Vacuumteknik A/S, Roy, Denmark) allowing the residual oxygen in the packs to be calculated. The vacuum + N_2 treatment was as follows: (i) a vacuum was drawn as above; (ii) the vacuum was reduced to 500 mbar absolute with N_2. The bags were then immediately heat-sealed. On the following day, after first opening each bag (iii) the vacuum was reduced to 50 mbar absolute with N_2, followed by heat-sealing. The objective was to allow time for the vacuole air to equilibrate with the N_2 and to replace the air-N_2 mixture with N_2 in step (iii).

Fat globule diameter

The fat globule dia of the emulsions was measured with a Malvern Mastersizer X (Malvern Instruments, Malvern, U.K.) using an MSX15 small-volume sample presentation unit. The instrument uses an approximation of the Mie scattering theory, which utilizes the refractive index of the dispersed phase and its absorption. A relative refractive index (n_{oil}/n_{water} = 1.095) and an absorption value of 0.1 were used in the calculations. A 2-mW He-Ne laser beam (633 nm) and a 300 RF lens (size range 0.05 to 879 m) were used for the measurements. The results were recorded as the volume-weighted dia of the lower decile of the number of the fat globules D(v, 0.1), the volume weighted median dia D(v, 0.5) and the volume weighted dia D(v, 0.9) of the upper decile of the number of fat globules. However, only the D(v, 0.9) value was reported because it was found to be the most sensitive indicator of homogenization efficiency and emulsion stability. One sample of each diluted emulsion was repeatedly measured until an equilibrium value was reached. For the reconstituted powder fat globule size, the powders were suspended (10% w/w) in deionized water at 60 °C.

Powder particle diameter

The particle dia of the powders was measured with the Malvern Mastersizer as described above. The powders were suspended in propan-2-ol and sonicated for 2 min before each determination.

Vacuole volume

The vacuole volume is determined by the difference between the solids density of the powder components and the particle density or density of the air-free powder. The solids density is calculated from the density contribution of each of the powder components. The particle density of the powders was measured with a pycnometer (AcuPyc 1330, Micromeritics, Norcross, Ga., U.S.A.). The method uses helium to measure the particle density of an air-free volume of a known weight of powder (Niro Atomizer A/S, 1978a). The vacuole volume is then defined as:

$$= [(100/\text{Particle density}) - (100/\text{Solids density})]$$

Table 2—Estimates of main effects and interactions of the input variables on the properties of the emulsions

	Term	Emulsion D(v,0.9) μm	Sig[3]	Reconstituted emulsion D(v,0.9) μm	Sig
		Box-Cox transformed values (λ = -1.00)		No transformation	
	Constant[2]	-0.31	—	0.64	—
Linear[1]	1 Homogenization pressure (MPa) × passes	-1.41	***	-1.85	***
	2 Vacuole volume (mL)	-0.42	***	-1.95	***
Interaction	3 [Homogenization pressure (MPa) × passes] × vacuole volume (mL)	—	NS[4]	—	NS
Quadratic	4[Homogenization pressure (MPa) × passes]^2	0.37	***	1.13	**
	5 Vacuole volume (mL)^2	—	NS	—	NS
Block	6 Packaging [vacuum]	—	NS	-0.33	*
	Res SD	0.08	—	0.50	—
	Rep SD	0.07	—	0.26	—

[1]A linear effect X implies a change in response in the range c ± 0.5X, where c is the constant.
[2]The constant is the response at the centered value of all the variables.
[3]Significance.
[4]Not significant.

Measurement of free fat, surface fat and extraction depth

The free fat content of the powders was determined by extraction with CCl_4 (Niro Atomizer 1978). Measurements of the surface composition of the powders were carried out by electron spectroscopy for chemical analysis (ESCA) (Fäldt and others 1993) at the Institute for Surface Chemistry, Stockholm, Sweden. In this procedure, samples are placed under very high vacuum (10^{-7} torr) in an AXIS HS photoelectron spectrometer (Kratos Analytical, Manchester, U.K.), where they are irradiated with X-ray photons of a well-defined energy. This causes a complete transfer of the photons' energy to an atomic or molecular orbital electron. Where the electron binding energy is lower than the photon energy, the electron is emitted from the atom with a kinetic energy equal to the difference between the photon energy and the binding energy minus the spectrometer work function. Since the total binding energy is characteristic for each element and orbital, an analysis of the emitted photoelectrons allows an identification of the elements of the near-surface region (about 10 nm depth). Each result is a mean of 3 analyses. The extraction depth d of the free fat of the powder particles was calculated according to Fäldt and others 1996 as

$$d\ (\mu m) = r\ (1-(1-(\Phi free\ fat/\Phi fat)^{1/3})$$

where r is the powder particle radius and $\Phi free\ fat/\Phi fat$ is the volume fraction of free fat of the total fat.

Off-flavor development during storage

Reconstituted powders were examined for off-flavor at 4-wk intervals during storage at 4 °C. The powders were made up to 8% total solids in deionized water. A maximum of 4 milks, randomly coded was presented to a trained taste panel of at least 7 members (Stone and Sidel 1993). There were no significant visual differences between the samples. Each panel member was required to identify 8 of the following 11 samples, with 75% success; sour (citric acid 0.01, 0.02, and 0.04, bitter (caffeine 0.01, 0.03, and 0.05%), sweet (sucrose 1 and 2%), salt (sodium chloride 0.1 and 0.2%) and water. Preliminary taste panel training sessions were used to identify the off-flavors and agree on the off-flavor terms. The panel defined the off-flavors of the reconstituted milks as being fishy, painty and metallic. The painty and metallic off-flavors increased during storage, but the fishy off-flavor did not and was treated separately. During subsequent assessment of the stored powders, the reconstituted milks were given a score for fishy, painty, and metallic on a scale of 0 to 100. The mean value for each off-flavor was recorded. Reconstituted milks with a mean (painty + metallic)/2 score < 10 were considered mild, 20 to 30 acceptable and 40 to 50 objectionable by the panel. A score of 25 was therefore chosen as the shelf life end-point. Twelve powder trials were carried out and 9 were replicated once (total 21 trials; see Statistical analysis).

Microscopy of powders

A small amount (about 2 mg) of powder was placed on a microscope slide and gently mixed with silicone oil (Sigma-Aldrich, Dublin, Ireland). Powder samples were analyzed using a Zeiss LSM 310 confocal laser scanning microscope (Carl Zeiss Ltd., Welwyn Garden City, Herts., U.K.) operating in transmitted light mode. Images (8 bit, 512 × 512 pixels) were acquired using 633-nm laser light-bright field illumination. Powders were examined using 10 × and 20 × objectives and 4 images were acquired at each magnification making a total of 8 images per sample. The structures of the powders and vacuoles were clearly visible, the latter appearing as black-ringed areas within the spray-dried powder particles.

Image analysis of vacuole volume

Digital images of the powders were analyzed using a Kontron KS400 image analysis software package (Imaging Assoc. Ltd., Oxford, U.K.). A macro was written to measure the vacuole volume as a percentage of the total spray-dried powder area.

Statistical analysis

The main experiment was statistically designed with 2 continuous variables (homogenization pressure × number of passes and vacuole volume) and 1 block variable (packaging treatment). The generation of the quadratic design, regression analysis and production of plots were carried out using ECHIP™, a statistical software package (ECHIP, Inc., Hockessin, Del., U.S.A.). The algorithm used (Wheeler 1989) selected a minimum of 12 trials at low, medium, and high levels of each continuous variable and a minimum of 5 replicated trials. In fact, 9 of the trials were replicated once (total

Table 3—Estimates of main effects and interactions of the input variables on the properties of the powders

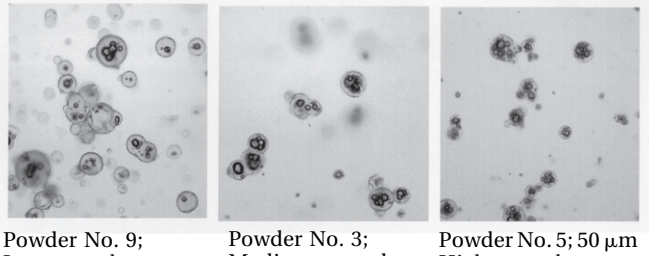
	Term	Free fat (g/100g fat)	Sig[3]	Surface fat (%)	Sig	Extraction depth (μm)	Sig
		Aranda-Ordaz transformed values (λ= 0.00)		No transformation		Box-Cox transformed values (λ = -0.19)	
	Constant[2]	-13.0	-	50.4	-	-0.106	-
Linear[1]	1 Homogenization pressure (MPa) × passes	-11.4	***	-4.8	*	-0.025	***
	2 Vacuole volume (mL)	-	NS[4]	-18.7	***	-0.009	**
Interaction	3 [Homogenization pressure (MPa) × passes] × vacuole volume (mL)	9.1	***	-5.9	*	-	NS
Quadratic	4[Homogenization pressure (MPa) × passes]^2	-	NS	-	NS	0.012	***
	5 Vacuole volume (mL)^2	-13.1	***	-	NS	-	NS
Block	6 Packaging [vacuum]	-1.8	*	-	NS	-	NS
	Res SD	2.72	-	3.29	-	0.004	-
	Rep SD	1.84	-	2.45	-	0.003	-

[1]A linear effect X implies a change in response in the range c ± 0.5X, where c is the constant.
[2]The constant is the response at the centered value of all the variables.
[3]Significance.
[4]Not significant.

21 trials), as outlined in Table 1. The replicate S.D. (Rep SD, Tables 3 and 4) gives a measure of the replicate error, while residual S.D. (Res SD) is a measure of the model error. The F-test was used to compare the residual S.D. to the replicate S.D. Lack-of-fit of the model to the data is indicated when the residual S.D. is about twice the replicate S.D. in the particular case of a quadratic design for 5 degrees of freedom. If more than the minimum number of replicates or trials are carried out, lack-of-fit will be indicated at a lower ratio of residual S.D. to replicate S.D. Significance of a variable is detected when the effect reaches roughly twice the residual S.D. in the same circumstances. Some of the responses fitted the quadratic model poorly. In these cases, a Box-Cox transformation of the data was used (Wheeler 1989):

$$Y = \frac{y^{\lambda} - 1}{\lambda}$$

where Y is the transformed value and y is the original value. The exponent λ was chosen iteratively to give the best fit of the data. Where values of a response such as free fat are expressed as g/100 g (bounded on 2 sides), the Aranda-Ordaz transformation of the data was used (Wheeler 1989). The transformation uses the intermediate variable $p = y/(1 - y)$, where y is the proportion of free fat. The transformation is $Y = 0.5 \log p$, where $\lambda = 0$, as was found for free fat in our study. The exponent λ was chosen iteratively to give the best fit of the data.



Powder No. 9; Low vacuole volume using SMP

Powder No. 3; Medium vacuole volume using Ca caseinate

Powder No. 5; 50 μm High vacuole volume using Na caseinate

Figure 1—Transmitted laser light micrographs of low, medium and high vacuole volume microencapsulated fish oil powders.

Results

Trials were first carried out to vary the vacuole volume of the fish oil powders in order to evaluate the effect of vacuole volume on the oxidation of the fish oil powders during storage. Typical values for vacuole volume on drying a sodium caseinate-stabilized emulsion using the Lab dryer and 2-fluid nozzle were 21 to 25 mL/100 g powder.

A novel process (Keogh and others 1998), developed during the course of the work, set out to prepare powders with varying levels of vacuole volume using an emulsifying protein, namely casein, with different levels of aggregation. This is based on the hypothesis that the greater the degree of aggregation of the protein emulsifier, the lower should be the level of foaming and thus vacuole volume. The level of aggregation of casein increases from sodium caseinate (low), calcium caseinate (medium) to micellar casein (high). It was, therefore, decided to use these 3 types of casein to evaluate their effect on vacuole volume and oxidation during storage. It was found that the vacuole volume level of the powders could be varied from approximately 21 to 14 to 7 mL/100 g powder, using sodium caseinate, calcium caseinate, and skim milk powder, respectively. The disadvantage is that vacuole volume is confounded with other properties of the caseins such as degree of aggregation, porosity, and antioxidant properties.

Main experiment

The effect of emulsion homogenization pressure × number of passes (15 MPa × 1, 30 MPa × 3 and 50 MPa × 5 passes), vacuole volume (7, 14 and 21 mL/100 g powder) and packaging treatment (vacuum and vacuum + N_2) on the off-flavor score of fish oil emulsions after homogenization and of powders stored at 4 °C was studied in a statistically designed experiment as described above. The trial number and treatment conditions of each emulsion are shown in Table 1. Thus, a vacuole volume of 7 is equivalent to skim milk powder; 14 is equivalent to calcium caseinate and 21 is equivalent to sodium caseinate. The vacuole volume values will be used to report the results. The vacuole volume values varied by approximately ± 2 mL/100 g powder around these mean values and actual values for each powder were used in the design. In this way, the actual vacuole volumes obtained were used as the experimental input values.

The vacuole area of each powder (as % of total area of the particles) was also quantified microscopically for confirma-

Table 4—Estimates of main effects and interactions of the input variables on the reconstituted powder off-flavor scores and shelf-life

		(Metallic + Painty)/2 off-flavor score						Shelf-life	
	Term	8 weeks	Sig[3]	16 weeks	Sig	24 weeks	Sig	(weeks)	Sig
		No transformation		Box-Cox transformed values (λ= 0.5)		No transformation		No transformation	
	Constant[2]	10.1		16.8	-	39.6	-	22.2	-
Linear[1]	1 Homogenization pressure (MPa) × passes	2.1	*	-	NS	-	NS	4.0	**
	2 Vacuole volume (mL)	7.2	***	-	NS	-	NS	-4.4	*
Interaction	3 [Homogenization pressure (MPa) × passes] × vacuole volume (mL)	-	NS[4]	-	NS	-	NS	-5.2	*
Quadratic	4[Homogenization pressure (MPa) × passes]^2	-	NS	3.7	*	-9.4	*	-	NS
	5 Vacuole volume (mL)^2	-3.4	*	-5.2	*	-	NS	-	NS
Block	6 Packaging [vacuum]	0.8	*	-	NS	-	NS	-	NS
	Res SD	1.45	-	2.96	-	7.17	-	2.28	-
	Rep SD	1.39	-	2.15	-	4.30	-	1.89	-

[1]A linear effect X implies a change in response in the range c ± 0.5X, where c is the constant.
[2]The constant is the response at the centered value of all the variables.
[3]Significance, [4]Not significant.

tion purposes. A correlation coefficient of 0.94 was found between the pycnometer and microscope image analysis data. Figure 1 shows laser light micrographs of low, medium and high vacuole volume microencapsulated fish oil powders made, respectively, with SMP, calcium caseinate and sodium caseinate. The vacuole area of these 3 powders was 7.0, 23.2 and 36.6% of the total area occupied by the powder particles, as estimated by image analysis.

The effects of the 3 input variables outlined above on selected properties of the emulsions[emulsion globule size D(v, 0.9) before drying, reconstituted emulsion globule size D(v, 0.9)] are shown in Table 2 and on the powders (free fat, surface fat and extraction depth) are shown in Table 3. The reconstituted powder off-flavor at 8, 16 and 24 wks and shelf life (wks to reach an off-flavor score of 25) of the powders are shown in Table 4.

Emulsion fat globule size

Table 2 shows that the homogenization pressure × passes (term 1) significantly ($p < 0.001$) decreased the emulsion fat globule size D(v, 0.9) value by 1.41 transformed units = 2.07 μm. When the data are transformed, the data in the table for that output variable are presented in transformed units. The data on the figures are presented in physical units, where they can be read directly. Figure 2 shows the decrease was from 2.73 to 0.66 μm as the homogenization pressure × passes changed from the low to the high level at the centered or mean values of the other input variables. The vacuole volume (term 2) also decreased ($p < 0.001$) the D(v, 0.9) value by 0.42 transformed units = 0.17 μm. The effect of homogenization pressure × passes (term 4) was also curvilinear ($p < 0.001$), especially above 40 MPa under the current conditions, as shown on Figure 2. The D(v, 0.9) value exceeded 1.0 μm below a homogenization pressure of 28 MPa using skim milk powder and below a pressure of 23 MPa using sodium caseinate.

Reconstituted emulsion fat globule size

The reconstituted emulsion globule size D(v, 0.9) value decreased by 1.85 μm ($p < 0.001$) as the homogenization pressure × passes increased and by 1.95 μm ($p < 0.001$) as the vacuole volume increased (Table 2). The effect of homogenization pres-

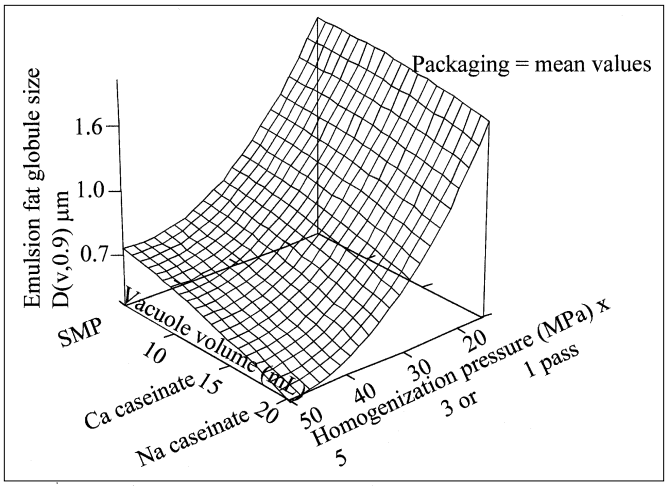


Figure 2—Effect of homogenization pressure and casein type or vacuole volume on the emulsion fat globule size D(v, 0.9).

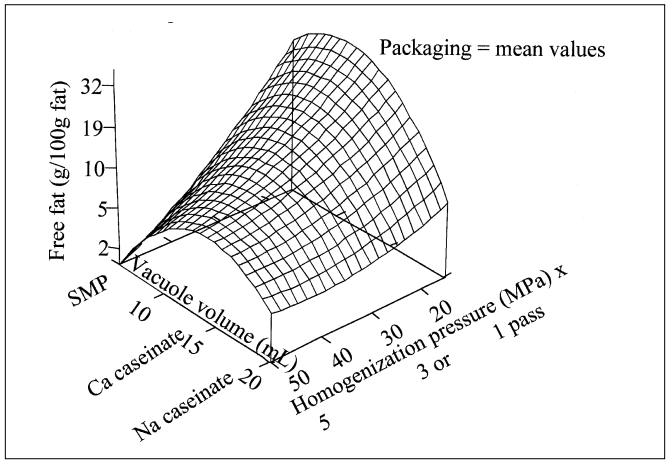


Figure 3—Effect of homogenization pressure and casein type or vacuole volume on the powder free fat.

sure × passes (term 4) was curvilinear ($p < 0.01$). The D(v, 0.9) value of the reconstituted powders which had been packaged under vacuum (term 6, Table 2) was also significantly lower ($p < 0.05$) by 0.33 μm, compared to vacuum + N_2 packaging.

Free fat

Table 3 shows that there was a significant interaction ($p < 0.001$) between the effects of homogenization pressure × passes and vacuole volume (term 3) on the free fat, as shown on Figure 3. The homogenization pressure × passes (term 1) reduced the free fat ($p < 0.001$) and the effect of vacuole volume (term 5) was curvilinear ($p < 0.05$). At the low vacuole volume (using SMP), the free fat decreased from 24.5 to 2.0 g fat/100 g fat as the homogenization pressure × passes increased, whereas at the high vacuole volume (using sodium caseinate), the free fat decreased insignificantly from 7.6 to 4.8 g fat/100 g fat. A response of 1.5 × (residual S.D.) = 6.1 physical units indicates significance for the linear effect of free fat. At the low homogenization pressure × passes, the free fat increased from 7.6 to 24.5 g fat/100 g fat as the vacuole volume decreased, whereas at the high homogenization pressure × passes, the changes in free fat were not of practical significance. The free fat of the block of powders packaged under vacuum (term 6, Table 4) was also significantly lower ($p < 0.05$) by 1.8 transformed units = 5.9 g free fat/100 g fat, compared to vacuum + N_2 packaging.

Surface fat

Increasing homogenization pressure × passes and vacuole volume decreased the surface fat (%) of the powder particles by 4.8% ($p < 0.05$) and 18.7% ($p < 0.001$), respectively (Table 4). There was a significant interaction ($p < 0.05$) between the effects of homogenization pressure × passes and vacuole volume, as shown in Figure 4. At the high vacuole volume (using sodium caseinate), increasing the homogenization pressure × passes decreased the surface fat from 47.4 to 37.5%, whereas at the low vacuole volume (using SMP), increasing the homogenization pressure × passes did not alter the surface fat significantly.

Extraction depth

Table 4 shows that increasing the homogenization pressure × passes reduced the extraction depth by 0.025 transformed units = 0.029 μm. Increasing vacuole volume also reduced the extraction depth to a lesser extent by 0.009 transformed units = 0.006 μm.

Metallic/painty off-flavor of the powders during storage

Table 4 shows that as the homogenization pressure × passes and vacuole volume increased, the metallic/painty off-flavor at 8 wks increased significantly by an off-flavor score of 2.1 ($p < 0.05$) and 7.2 ($p < 0.001$), respectively. The effect of vacuole volume^2 (term 5; ^2 indicates the squared term) on the score at 8 wks was slightly curvilinear (Figure 5), and the effect of packaging under vacuum (term 6) compared to vacuum + N_2 was to increase the mean off-flavor score by 0.8. However, the latter effect is not of practical significance.

The effects of homogenization pressure × passes^2 and vacuole volume^2 on the metallic/painty off-flavor score at 16 wks were significantly curvilinear ($p < 0.05$ in both cases). The effect of homogenization pressure × passes^2 on the metallic/painty off-flavor score at 24 wks was also significantly curvilinear (Figure 6). Figure 6 shows that the level of off-flavor was significantly reduced at the highest homogenization pressure when the low-vacuole-volume powder (SMP) was used.

On the assumption that the occluded air is not reduced by the vacuum treatment, air levels of 21, 14 or 7 mL air/100 g powder corresponding to 4.4, 2.2 or 1.1 mL oxygen/100 g powder, or 18, 9 or 4.5 mmoles oxygen, respectively, remain in the vacuoles, according to the ideal gas law PV = nRT (for P = 100 kPa and R = 8.314 J/(mol K). The interstitial air is reduced by the vacuum treatment. The number of moles of oxygen n in a residual vacuum of 4 mbar absolute in a 200 mL pack containing 100 g powder of bulk density 0.50 and interstitial volume of air in powder 120 mL at 20 °C corresponds to P = 400 Pa, V of $O_2 = 120 \times 0.21/10^6$ m^3 and T = 293 K. Thus $n = (400 \times 120 \times 0.21/8.314 \times 293 \times 10^6) = 0.004$ mmoles O_2, which is negligible, relative to the vacuole air. Assuming that sand eel oil triglycerides contain fatty acids with 19 carbon atoms and 2 double bonds per fatty acid, the average molecular weight of sand eel oil triglycerides is 928.

Thirty g oil/100 g powder corresponds to 32.33 mmoles

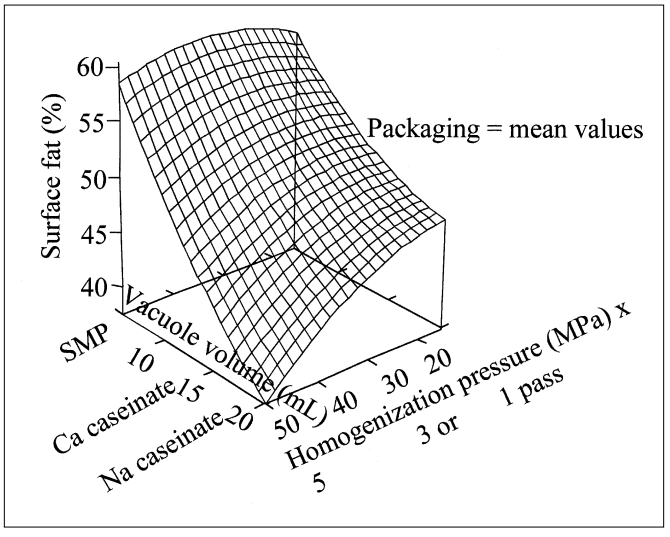


Figure 4—Effect of homogenization pressure and casein type or vacuole volume on the powder surface fat.

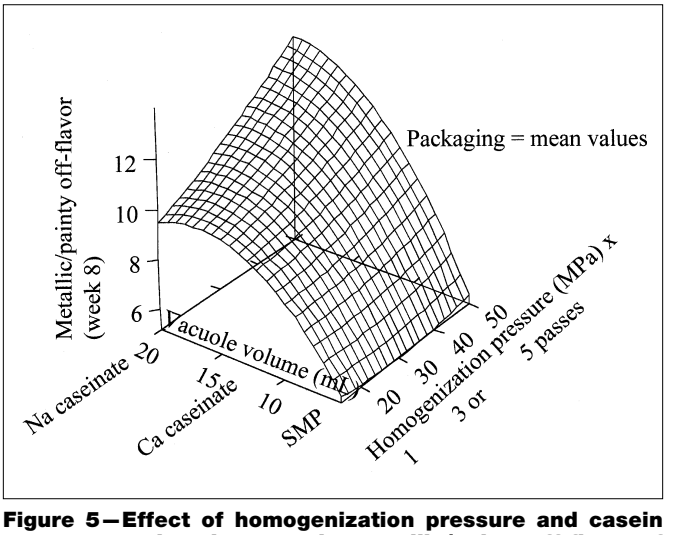


Figure 5—Effect of homogenization pressure and casein type or vacuole volume on the metallic/painty off-flavor of powders (week 8).

expressed as triglyceride. Thus, the molar ratio of oxygen to triglyceride is about 1:2, 1:4 and 1:8, respectively. These ratios indicate that the amount of oxygen available in the vacuoles is much lower than theoretically needed to oxidize all double bonds, which is a molar ratio of oxygen to triglyceride of 6:1, assuming 6 double bonds per triglyceride.

Shelf life (wks to reach off-flavor score of 25)

Table 4 also shows that, as the homogenization pressure increased, the shelf life of the powders significantly ($p < 0.01$) increased by 4.0 wks. As the vacuole volume decreased, the shelf life of the powders also significantly increased by 4.4 wks ($p < 0.05$). There was a significant interaction ($p < 0.05$) between the homogenization pressure and vacuole volume as shown in Figure 7, which shows that at the low vacuole volume, the shelf life increased from 23 wks at a homogenization pressure of 15 MPa × 1 pass to 31 wks at 50 MPa × 5 passes. At the high vacuole volume, the shelf life was not significantly affected by the homogenization pressure.

Discussion

THE MAIN OBJECTIVE OF MICROENCAPSULATION WAS TO MAXIMIZE protection of the fish oil from oxidation during storage of the powder. Preliminary work by us showed that homogenization conditions of 45 MPa × 4 passes resulted in fat globule D(v, 0.5) values of 0.48 μm and surface fat levels of 48%, which were not sufficiently low to prevent off-flavor development during storage. Interstitial air content and vacuole volume of powders partly reflect air entrapment during processing and drying. Since interstitial air, or air spaces between particles, is mostly removable by vacuum-packaging, attention was focused on in-process measures to reduce air entrapment in the powder particles, expressed as vacuole volume. Vacuole volume is affected by atomization conditions, concentrate total solids and foaming characteristics of the concentrate. The presence of oil depresses the foaming tendency of concentrates and a pneumatic 2-fluid nozzle atomizer would be expected to give higher vacuole volumes than either disc and pressure nozzle types.

Attempts were first made to reduce the vacuole volume of the powders by increasing the total solids of the emulsions before drying. A total solids of 30% is below the optimum (40 to 45%) total solids used for drying whole milk concentrates. However, viscosity of the concentrates limited increases in total solids, if the proportion of fat:total solids in the powders was to be maintained at 1:3. Apart from raising total solids and using pressure nozzle atomization, other methods recommended for vacuole volume reduction include the use of high-heat treatment as applied to the drying of skim milk concentrate (Pisecky 1978). Heat denaturation of the whey proteins present is believed to reduce their tendency to foam through interaction with casein micelles. This does not apply here, since whey proteins are not present in the emulsions made with caseinates. A second possibility is to maximize the level of free fat, since fat when free has foam-depressant properties (Verhey 1972). This method was unsuitable, since free fat is known to be more likely to oxidize (Buma 1971a).

The novel approach adopted was the subject of a provisional patent (Keogh and others 1998). By using increasingly aggregated types of casein in the order:

sodium caseinate < *calcium caseinate* < *micellar casein*

it was possible to reduce the corresponding vacuole volume levels in the powders from approximately 21 to 14 to 6 mL/100 g powder. Emulsion oil globule size (before and after drying), powder surface fat and extraction depth increased as vacuole volume decreased, but shelf life under the same conditions increased. This indicates that vacuole volume or the level of occluded air in the powders had a greater effect on the oxidation of the fish oil powders during storage than these physicochemical properties. It was calculated that the amount of oxygen available in the vacuoles of the samples was much lower than theoretically needed to oxidize all double bonds and that the oxygen in the interstices after vacuum treatment was negligible. It is important to note that these calculations only mean that for rapid oxidation, oxygen should not be restricted much below the theoretical molar equivalence, because a decrease in oxygen may lead to retardation of oxidation. However, only very small amounts of oxygen are necessary to initiate the autoxidation process. It is impossible to predict the minimum amount of oxygen necessary to give rise to detectable oxidation products in these complex heterogeneous matrices. In these powders, oxidation is heterogeneous since some triglyceride molecules are probably much more exposed to the occluded air than others. Therefore, although the amount of oxygen available is not high, a part of the encapsulated oil can be in direct contact with the occluded air and develop detectable off-flavor, indicating the end of shelf life. However, when the powder sample is extracted and the total encapsulated oil analyzed for chemical indicators of oxidation, chemical changes may not be detected because of the high proportion of unoxidized oil. In other words, the off-flavor results show indirectly that there was enough oxygen present to give rise to sufficient hydroperoxides necessary for the release of volatiles detected by the panel test during storage (Marquez-Ruiz 2000, personal communication).

However, vacuole volume is confounded in the experimental design with properties of casein in the final powders such as state of aggregation, porosity and antioxidant efficiency. As homogenization pressure increased at low vacuole volume (using SMP), only the free fat of the powders decreased in parallel with the powder off-flavor. The correlation coefficient r for free fat and shelf life was −0.31 (n = 21, not significant). Buma (1971b) showed that free fat was strongly related (r = 0.94) to particle porosity. Lower free fat levels should also indicate lower porosities in the current work. Thus, the reduction in the rate of oxidation may be partly due to the lower porosity of the more aggregated types of casein used. No indications in the literature have been found to suggest this occurs for caseins. However, it has been shown that the levels of encapsulating whey protein isolate and fat affected particle porosity (Moreau and Rosenberg 1998). With modified starch, it has been shown that combinations of larger and smaller molecules give rise to a reduced rate of oxidation (McGlinchey 1997). The effect of vacuole volume on the rate of oxidation could be isolated by varying vacuole volume using the same type of casein. We feel that under vacuum-packaging conditions with very low interstitial air, the rate of oxidation will be related more to occluded air. Conversely, when stored in air with very low vacuole volume, the rate of oxidation will be related to the proportion of the particle surfaces covered by fat. The effect of high porosity (and free fat) will be to allow air to diffuse from the interstices of the powder into the particles or from the vacuoles to the surface of the particles.

Maximum shelf life was estimated by extrapolation to be 36 wks at zero vacuole volume and a homogenization pressure of 50 MPa. Surface fat was lower at the high level of vacuole volume (using sodium caseinate) than at the low level of vacuole volume (using SMP). The correlation coefficient r

between surface fat and shelf life was + 0.33, showing that these variables were not inversely related. The extraction depth decreased equally at all levels of vacuole volume. Thus, only free fat was related to shelf life under our conditions. Work on these powder samples to be reported (Marquez-Ruiz 2000, personal communication) showed that the internal fat was more oxidized than the surface fat, reflecting their high levels of vacuole volume or occluded air.

The minimum D(v,0.9) values of the emulsions, before and after drying, and of the surface fat, each occurred at the highest homogenization pressure (50 MPa × 5 passes) and the highest vacuole volume (using sodium caseinate), while maximum shelf life was found using SMP. The changes in the levels of free fat and extraction depth with vacuole volume were not of practical significance at a homogenization pressure of 50 MPa × 5 passes. In addition, there was no significant change in surface fat at the low vacuole volume (skim milk powder) as the homogenization pressure increased. It is of some interest to note that the reconstituted droplet size and free fat values were higher in vacuum + N_2-treated powder samples than in vacuum-packed samples. For highly sensitive materials such as fish oil, this indicates that slight damage occurred to the powder samples during the second vacuum treatment. However, this did not result in any significant difference in the shelf life of the 2 packaging treatments.

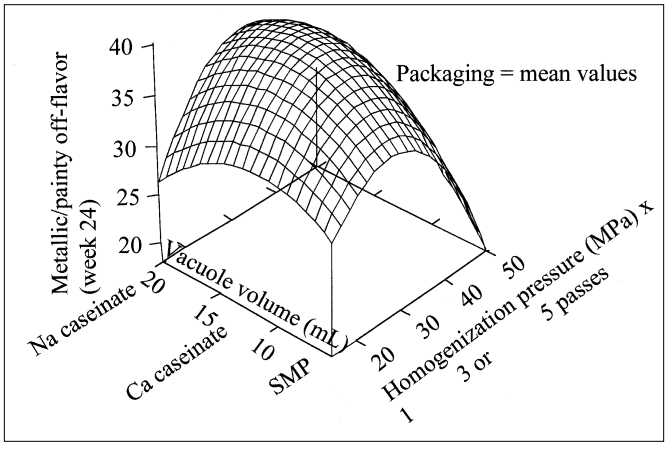


Figure 6—Effect of homogenization pressure and casein type or vacuole volume on the metallic/painty off-flavor of powders (week 24).

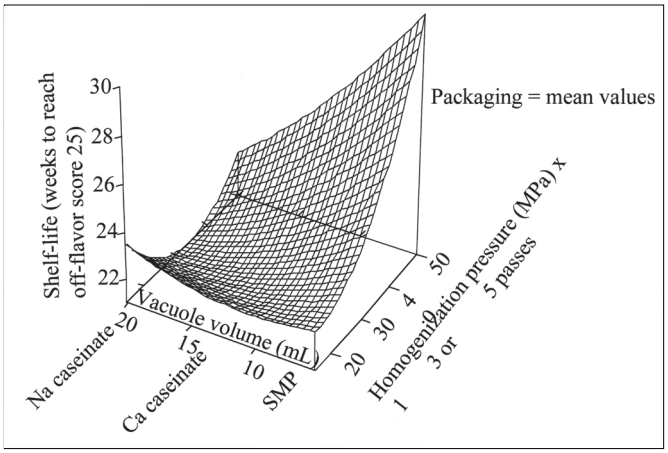


Figure 7—Effect of homogenization pressure and casein type or vacuole volume on the shelf-life (weeks to reach off-flavor score 25).

Conclusions

IT WAS POSSIBLE TO REDUCE THE VACUOLE VOLUME OF FISH oil powders after spray-drying using a 2-fluid nozzle from approximately 21 to 7 mL/100 g by changing the encapsulating protein in the emulsion from sodium caseinate to micellar casein (SMP). When the vacuole volume level was extrapolated to zero, a shelf life of 36 wks at 4 °C at high homogenization conditions was estimated. At a low vacuole volume using SMP, the shelf life increased as the homogenization pressure increased, but at the high vacuole volume using sodium caseinate, increasing homogenization pressure did not significantly affect the shelf life. Only the free fat values paralleled the shelf life at the low vacuole volume using SMP and high homogenization pressure. The main conclusion of the work is that a fish oil powder with a low level of off-flavor can be produced with a shelf life of 31 wks at 4 °C, using dairy ingredients alone as encapsulating agents.

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