Characterization of Fish Oil-in-Water Emulsions Using Light-Scattering, Nuclear Magnetic Resonance, and Gas Chromatography-Headspace Analyses

Zhiping Shen*[,a], Punsandani Udabage[a], Iko Burgar[b], and Mary Ann Augustin[a]

[a]Food Science Australia, Werribee 3030, Victoria, Australia, and [b]Commonwealth Scientific and Industrial Research Organisation (CSIRO) Manufacturing and Infrastructure Technology, Clayton, Victoria 3168, Australia

ABSTRACT: The effect of the molecular environment on the physical and oxidative properties of homogenized or microfluidized fish oil-in-water emulsions (5% w/w tuna oil in pH 7 phosphate buffer) stabilized by whey protein isolate (WPI, 1 or 5% w/w) or lecithin (2.5% w/w) was examined. Laser light-scattering measurements showed that WPI-stabilized emulsions had smaller particle sizes than lecithin-stabilized emulsions, and that higher pressures reduced the particle size. WPI afforded more protection against oil oxidation than did lecithin, as evidenced by the lower headspace propanal of emulsions as measured by GC-headspace analysis, despite the larger interface in WPI-stabilized emulsions. Reducing the concentration of WPI in emulsions from 5 to 1% decreased the oxidative stability of WPI-stabilized emulsions. The ^{1}H NMR transverse relaxation times (T_2) of FA chains in emulsion droplets stabilized by the same surfactants made by homogenization or microfluidization were different and not always related to particle size. The higher mobility (i.e., longer T_2) of the unsaturated parts of the FA chains within an oil droplet, compared with the saturated parts, suggests that the unsaturated components tended to stay in the core of the oil droplets. This experimental result supports the hypothesis reported in other literature that the more unsaturated FA are buried in the oil core of oil-in-water emulsions. The lack of a universal correlation between particle size and oxidation suggests that the mobility of particles in an emulsion has an influence on the rate of oxidation.

Paper no. J11057 in *JAOCS 82*, 797–802 (November 2005).

KEY WORDS: Fish oil-in-water emulsion, headspace (HS) analysis, lecithin, n-3 fatty acids, NMR, particle size, propanal, relaxation time (T_2), whey protein isolate.

Fish oil is a rich source of long-chain n-3 FA, and these FA are known to have a range of health benefits. Their potential role in reducing the risk of coronary heart disease, inflammatory disorders, and immune disorders has resulted in interest among food manufacturers in fortifying foods with long-chain n-3 FA (1,2). Because n-3 FA are polyunsaturated, and are therefore very susceptible to oxidation, they need to be protected from factors that promote oxidation (e.g., oxygen, light, free radicals, and pro-oxidants) during transportation and storage, and also when they are incorporated into manufactured food products. Oils containing n-3 FA may be incorporated into foods as bulk oils, microencapsulated powders, or aqueous emulsions (3–6).

When incorporated into manufactured food products, the oils are usually in the form of emulsions, in which they are stabilized by surfactants. However, the oxidation of the n-3 FA is still the major deteriorative reaction occurring in fish oil emulsions (7). The use of very high quality fish oils, with PV as low as 0.1 meq/kg oil, has enabled the preparation of fish oil-enriched milks without a detectable fishy off-flavor during the storage period (8). Other approaches used to improve the stability of n-3-rich oils in emulsions include changing the emulsifier type and concentration, altering the emulsion droplet charge, increasing the thickness of the emulsifier layer, or adding antioxidants and chelating agents (4,9–11).

Proteins and small-molecule surfactants have a strong tendency to adsorb at the oil–water interface in food oil-in-water emulsions (12). Proteins (e.g., whey proteins and caseins) and low-M.W. surfactants (e.g., lecithin, Tweens, and sucrose FA esters) have been used to stabilize n-3 oil emulsions. The protection afforded by the surfactants against oil oxidation depends on the ingredient source, the concentration, the pH of the emulsion, the methods used for the emulsification of the oil, and the type of n-3 oils used (13–16). The effects of the molecular environment (e.g., chemical structure of the lipids, oxygen concentration, antioxidants, electrical charge, and physical barriers to lipid oxidation) in food emulsions have been comprehensively reviewed (7). NMR techniques have been applied to determine the particle size of emulsions in a wide range of products, from cheese and margarine to crude oil emulsions, and to characterize other emulsion properties such as the diffusion and flow in emulsions, the diffusion of concentrated emulsions, and the shelf life and stability of emulsions (17). ^{1}H NMR has been employed to measure the chemical shift values of specific oxidation products of ethyl docosahexaenoate with and without an antioxidant (18) and the results are well correlated with the PV and conjugated dienes of ethyl docosahexaenoate detected by conventional analysis methods. The effect of the molecular environment, particularly the molecular mobility inside the oil droplet and in the aqueous continuous phase, on the physical and chemical stabilities of n-3 oil emulsions stabilized by different types of surfactants has rarely been investigated. A correlation between an NMR characterization

*To whom correspondence should be addressed at Food Science Australia, 671 Sneydes Rd., Werribee 3030, VIC, Australia.
E-mail: Zhiping.Shen@csiro.au

of the emulsions and other conventional analysis methods has not been reported.

In this study, a high-M.W. surfactant, whey protein isolate (WPI), and a low-M.W. surfactant, lecithin, were selected to make fish oil-in-water emulsions dispersed by either homogenization or microfluidization (MF) in order to compare their physical and chemical stabilities. These surfactants were chosen because they have commonly been used to stabilize fish oil emulsions. Laser light-scattering and headspace (HS) analyses of propanal were used to measure particle sizes and to assess oil oxidation, respectively. To further understand how the molecular environments affected the physical and chemical stabilities of the tuna oil-in-water emulsions, ^{1}H NMR techniques were employed to characterize the dynamics of the molecular environments of the oil droplets and in the continuous water phase of the emulsions by measuring the transverse relaxation time (T_2).

EXPERIMENTAL PROCEDURES

Materials. WPI and lecithin (Metarin P PCR Negative) were purchased from Murray Goulburn Co-op Co. Ltd. (Brunswick, Victoria, Australia) and Bronson and Jacobs Pty. Limited (Homebush, New South Wales, Australia), respectively. Propanal (>98% purity) was purchased from Sigma-Aldrich Pty. Ltd. (Sydney, Australia). Tuna oil (HiDHA 25N Food) was purchased from NuMega (Queensland, Australia).

Emulsion preparation. For WPI-based emulsions, an appropriate amount of WPI powder was dispersed in 0.05 M phosphate buffer (pH 7.0) and stirred for 30 min at ambient temperature (*ca*. 22°C). Tuna oil was removed from storage at −18°C and melted completely in a water bath at 60°C prior to making the emulsion. The oil was dispersed in the protein solution by stirring mechanically for 15 min at 60°C to make a tuna oil-in-water emulsion (pH 7, phosphate buffer) containing 5% (w/w) tuna oil and 1 or 5% (w/w) WPI. For lecithin-based emulsions, an appropriate amount of lecithin powder was dispersed in the tuna oil at 60°C and the mixture was then added to 0.05 M phosphate buffer solution and stirred for a further 15 min at 60°C to make a final formulation containing 2.5% (w/w) lecithin and 5% (w/w) tuna oil.

Emulsion processing. The aforementioned mixtures were passed through a two-stage homogenizer at a first-stage pressure of 17.2 MPa and a second-stage pressure of 3.4 MPa at 50°C or through a microfluidizer at 40 or 120 MPa with one pass at 50°C. All processed emulsions were given ultra-high-temperature treatment at 140°C for 3 s. The emulsion solutions were stored at 25°C and removed from storage for testing at 24 h and at 2, 4, and 8 wk.

Particle size measurement. The particle size distribution (PSD) of the emulsions stored at 25°C for 24 h or 2, 4, or 8 wk were determined using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, Worcestershire, United Kingdom). A particle refractive index of 1.456 and an absorption of 0 were assumed for all emulsions. Samples were stirred and added to circulating distilled water to obtain an obscuration of *ca*. 15%. All measurements were carried out in triplicate at room temperature (*ca*. 22°C).

Propanal analysis. A standard series of 0.1, 0.5, 1, 5, and 10 μg propanal/g deionized water was freshly made on the test day. In this study, a water solution of standard propanal was used to calibrate the propanal concentration of the emulsion samples. Five milliliters of the standard propanal solution or emulsion was pipetted into a 20-mL HS GC vial. The vial was sealed and equilibrated at 60°C for 30 min in an HS autosampler. Approximately 0.6 mL of the HS vapor was directly transferred into the GC column (DB1, 30 m × 0.25 mm i.d., 5 μm film thickness; Agilent Technologies Australia, Melbourne, Australia). The GC column was programmed from 60°C, increasing at a rate of 3°C/min to 75°C, then at 5°C/min to 90°C, and finally at a rate of 20°C/min to 220°C, with a 10-min hold; the column head pressure was maintained at 120 kPa. The FID temperature was 240°C. The lowest detectable concentration of propanal was *ca*. 0.03 μg propanal/g water. Triplicate sample analyses were carried out.

NMR analysis. Proton relaxation measurements were made using an NMR spectrometer, (Varian Unity Plus 300 MHz WB, wide bore). Approximately 3 mL of the emulsion sample was placed in a 10-mm (i.d.) NMR tube. The transverse relaxation time (T_2) of protons was measured by using Carr, Purcel, Meiboom, and Gill (CPMG) pulse sequences: $90(x) - [\tau - 180(y) - \tau - \text{echo}]_n$. Acquisition parameters were set to a 90° pulse of 16 μs and a recycle delay of 2 s. The 90–180° pulse spacing (τ) was set to 72 μs. A total of four scans were collected after variable numbers of echoes for each measurement in an overall period after the first pulse of 1 to 200 ms. All measurements were performed at 20 ± 0.1°C. Relaxation curves obtained for the resonance peaks using CPMG sequences were analyzed using a single exponential curve fitting. When the proton chemical shift spectra from different molecules with different T_2 overlapped, the data were analyzed with a double exponential fit using Igor curve-fitting software if a single exponential fit did not match the data points. Igor software provides double exponential parameters and visible fitting to the experimental data. In standard proton and carbon NMR spectra, the chemical shifts were given in relation to the external standard trimethylsilane as a reference. The water peak resonance at 4.8 ppm was also used as an alternative reference.

RESULTS AND DISCUSSION

Particle size. Typical PSD profiles and $D_{3,2}$ of the emulsions (freshly made) are given in Figures 1 and 2 respectively. The PSD data showed that emulsions processed by MF, stabilized with the two surfactants, were unimodal and had smaller particles compared with emulsions processed by homogenization. Homogenized emulsions had a major peak and a broad shoulder (Fig. 1), and other authors have also found that MF produced smaller and more homogeneous particles than did homogenized emulsions (19,20). There was the expected trend of decreasing particle size with increasing pressure (19), and our results agreed with the literature (Figs. 1, 2). The particle size

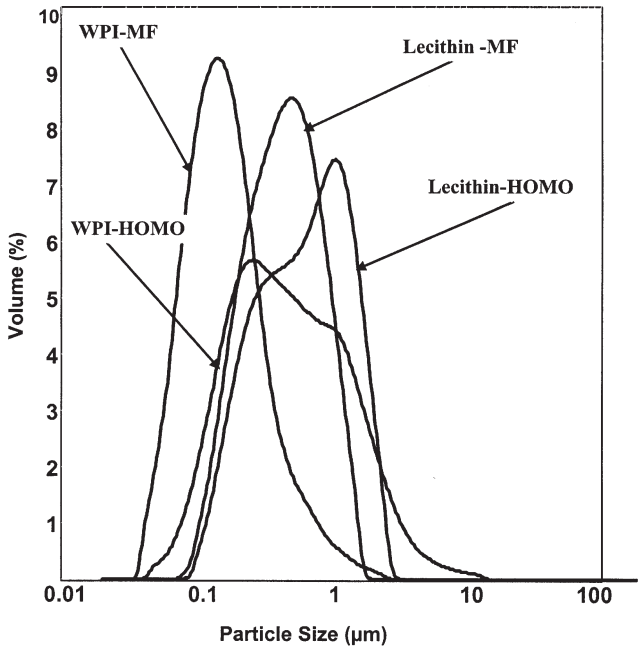

FIG. 1. Effects of surfactant type and processing on the particle size distribution (PSD) of freshly made fish oil-in-water emulsions (5% w/w oil) stabilized with 5% (w/w) whey protein isolate (WPI) or 2.5% (w/w) lecithin. Homogenization (HOMO) passes at 17.2 and 3.4 MPa, and one microfluidization (MF) pass at 120 MPa.

of all the emulsions did not change over an 8-wk period. The exception was the homogenized WPI-stabilized emulsion at later stages of storage, but the reason for this change is not understood.

The WPI concentration did not significantly affect the emulsion particle size, although there were slight decreases in droplet size with increasing WPI concentrations from 1 to 5% (Fig. 2). This is in line with the results of Faraji *et al*. (21), who reported that the mean particle diameter of emulsions (containing 10% w/w oil) homogenized at 13.8 MPa stabilized by WPI decreased from 0.59 to 0.46 μm when the concentration of WPI was increased from 0.25 to 1.5%.

The surfactant type had more of an effect on particle size. The particles in emulsions stabilized by 5% (w/w) WPI were smaller than those containing 2.5% (w/w) lecithin if the same emulsification process was used (Figs. 1, 2). Osborn and Akoh (11) reported that particle size distributions of emulsions (10% oil) stabilized by 0.5% WPI were unimodal, with a narrower distribution and smaller particle diameter ($D_{3,2}$) than emulsions stabilized by 0.5% sucrose FA esters. These results showed that the high-M.W. protein emulsifier was more effective than the low-M.W. surfactants for physical stabilization of the oil.

Oxidative stabilities measured by HS analysis of propanal. Aldehydes are considered to be one of a wide variety of different molecules responsible for the characteristic chemical and sensory properties of oxidized oils (7). For an oil rich in n-3 FA, propanal is an important indicator of oil oxidation. In this study, the HS propanal concentration in fish oil emulsions stabilized by WPI was always lower than that in lecithin-stabilized emulsions during storage (Fig. 3). This was also observed in our preliminary experiments, in which HS propanal values after 2 wk of storage were 0.82 and 0.32 μg/g emulsion, respectively, for microfluidized emulsions (40 MPa, 1 pass) with 1 and 5% (w/w) WPI and 0.88 μg/g emulsion for the emulsion containing 2% (w/w) lecithin. Separate experiments with homogenized and microfluidized emulsions (120 MPa, 1 pass) confirmed that the superiority of WPI over lecithin was maintained over a longer storage time (Fig. 3). After 8 wk of storage, the concentration of propanal in the HS of homogenized emulsions containing 2.5% lecithin was 5.3 times higher than in homogenized emulsions containing 5% WPI (Fig. 3). The partitioning of propanal between the oil phase, aqueous phase,

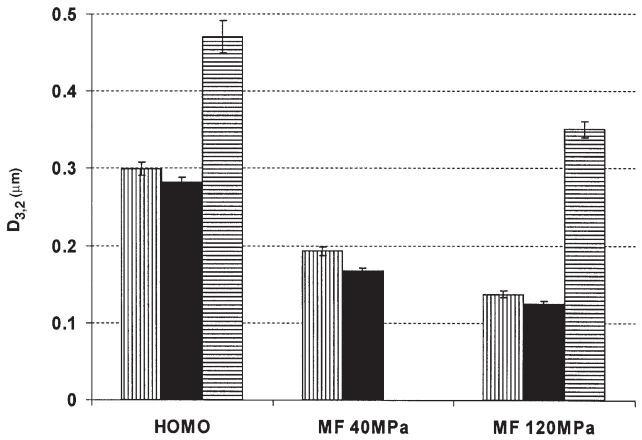

FIG. 2. PSD of freshly made fish oil-in-water emulsions as a function of different surfactants (WPI, lecithin) and different processing conditions. HOMO passes at 17.2 and 3.4 MPa, one MF pass at 40 or 120 MPa. Vertically striped bars, solid bars, and horizontally striped bars represent 1% WPI, 5% WPI, and 2.5% lecithin, respectively. (The lecithin emulsion was not processed at 40 MPa.) For abbreviations see Figure 1.

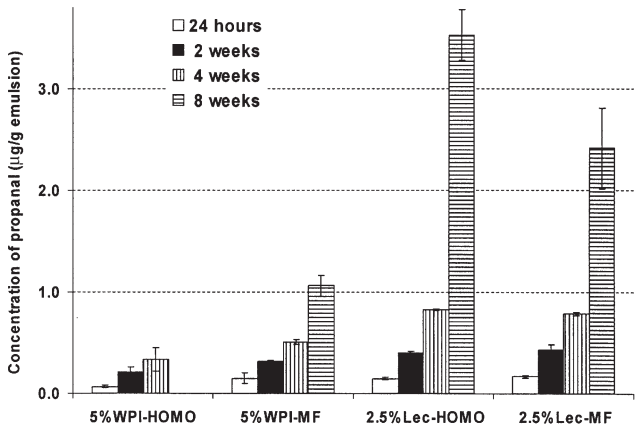

FIG. 3. Headspace analysis of propanal during storage at 25°C (triplicate analysis) of emulsions with different surfactants [WPI, lecithin (Lec)] and different processing conditions. HOMO passes at 17.2 and 3.4 MPa, one MF pass at 120 MPa. From left to right, bars represent 24 h, 2 wk, 4 wk, and 8 wk. (The 5% WPI-HOMO data set lacks a reading at 8 wk because the sample was contaminated by bacteria.) For other abbreviations see Figure 1.

and HS of different emulsions may affect the propanal detected in the HS. This will depend on the solubility of the propanal in the emulsions as well as the ability of the surfactant to interact with the propanal. Meynier *et al*. (22) reported that proteins have a tendency to bind aldehydes, particularly 2-alkenals. Increased interaction between whey protein and aldehydes occurs with an increased length of the aldehyde carbon chain (23), and for short carbon chain aldehydes (e.g., butanal and hexanal), binding increases with increased pH values. From our study, the HS propanal concentration of the WPI solution (5% w/w) was 80% that of a pure water solution containing the same propanal concentration. The interaction of lecithin with propanal was not examined in this study. We concluded from our results and the literature results just quoted that the HS propanal was not quantitatively related to the absolute amount of propanal formed when comparing emulsions with different binding capacities for propanal. However, the HS propanal was taken as a good indicator of the development of rancid odors in the oil. Hence, there was some uncertainty about the absolute amount of propanal developed, but only HS propanal relating to the odor of the emulsions could be compared. HS propanal is known to be linked to sensory properties. McClements and Decker (7) reported that WPI inhibits lipid oxidation in oil-in-water emulsions either at the emulsion droplet interface or in the aqueous phase. Osborn and Akoh (11) reported that WPI was a better antioxidative surfactant than the low-M.W. surfactant sucrose FA, an observation that is in line with our results. A higher concentration of WPI was more effective than a lower concentration of WPI for protection against oil oxidation, as evidenced by a significantly higher concentration of propanal in 1% WPI emulsions compared with 5% WPI emulsions produced by the same emulsification procedure (data not shown). Hu *et al.* (15) suggested that the interface of the emulsion droplets became saturated with 0.2% (w/w) WPI in an oil (containing 5% w/w oil)-in-water (pH 7 potassium phosphate buffer) emulsion homogenized at 34.4 MPa and that further increases in WPI left excess WPI in the continuous phase. Since WPI has antioxidative properties, it is possible that the excess

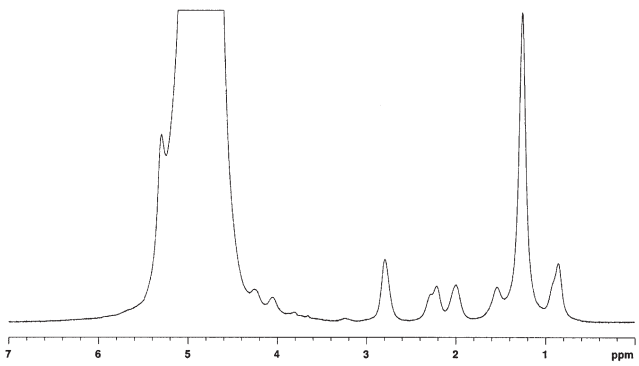

FIG. 5. Proton NMR spectrum of the fish oil emulsion (freshly made) stabilized by WPI in this study. The x axis represents the chemical shifts (ppm). For abbreviation see Figure 1.

WPI in the continuous aqueous phase contributed to the better oxidative stability of the oil (18). Although the concentrations and conditions were different in this study, one can expect that emulsions containing 5% (w/w) WPI would have more excess protein in the continuous phase than would 1% WPI emulsions. Another potential factor that could have contributed to the oxidative stability of the emulsions stabilized by 5% WPI was the thicker barrier of protein at the interface.

Molecular environment of emulsions assessed by NMR. NMR spectra provide information about the molecular environment. The proton spectra of tuna oil and the emulsions are given in Figure 4, 5, and 6, respectively. The pure tuna oil spectrum revealed nine resonance bands at 0.83, 1.22, 1.51, 1.98, 2.25, 2.77, 4.03, 4.21, and 5.28 ppm. These resonances were assigned to different parts of the oil molecules, in the FA chains and the glycerol backbone (24). In emulsions we observed similar proton spectra, but some resonances overlapped with the proton resonances of water (bands at 4.03, 4.21, and 5.28 ppm). The characteristic chemical shifts enabled the protons in water to be distinguished from most of the protons of the FA chains and the glycerol backbone. In this study, an NMR analysis was used to identify the environment of single chemical components, based on the fact that the NMR signals from protons in

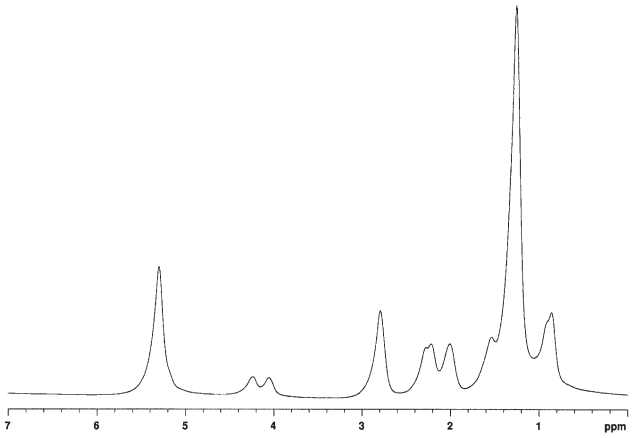

FIG. 4. Proton NMR spectrum of the fresh, pure fish oil used in this study. The x axis represents the chemical shifts (ppm).

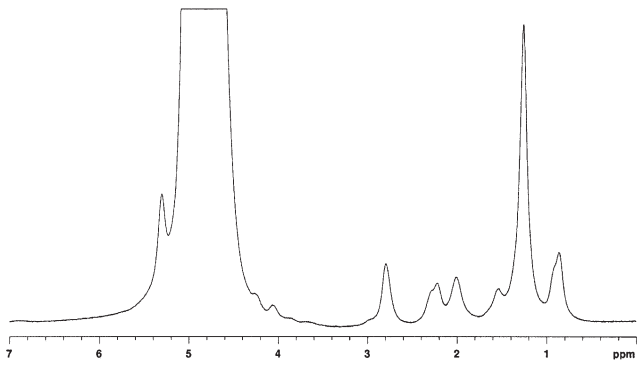

FIG. 6. Proton NMR spectrum of the fish oil emulsion (freshly made) stabilized by lecithin in this study. The x axis represents the chemical shifts (ppm).

TABLE 1
Transverse Relaxation Time (T_2) in Various Parts of the Emulsions Correlated with Particle Sizes and Propanal Data

Surfactant (% w/w)	Processing	[Propanal] at 4 wk (μg/g emulsion)	Initial $D_{3,2}$ (μm)	T_2 of –(C**H**$_2$)$_n$– in FA chains[a]	T_2 of =CH–C**H**$_2$–CH= in FA chains[a] (ms)	T_2 of H_2O[a]
WPI (5%)	Homogenization	0.34	0.35	163	263	76
WPI (5%)	Microfluidization	0.51	0.13	102	478	153
Lecithin (2.5%)	Homogenization	0.83	0.47	120	410	492
Lecithin (2.5%)	Microfluidization	0.78	0.35	105	430	612

[a]Chemical shifts of –(C**H**$_2$)$_n$– and =CH–C**H**$_2$–CH= resonance were at *ca.* ~1.3 and 2.8 ppm, respectively. NMR results are accurate to around 5–10%; pure oil had a T_2 of *ca.* 700 ms and pure water a T_2 of more than 1000 ms. WPI, whey protein isolate.

oil or water could be separated in the spectra and that they reflected the different molecular environments in the emulsion. In particular, the molecular dynamics were investigated by using standard transverse relaxation time measurements (T_2). In general, the relaxation time was strongly influenced by the environment and the mobility of the observed nuclei (25). Choi and Kerr (26) indicated that some T_2 values were less than 10 ms for water in starch samples, reflecting a strong interaction between water and starch. The T_2 of pure water was 2.0 s. Bulk liquid material had a long T_2, and material on the surface and at the interfaces had shorter T_2 as its motion was restricted.

Usually multiple exponential decay needs to be used in the analysis of proton relaxation curves when there is nonuniform mobility of protons. In our case, all proton spectra could be resolved for each of the main groups, such as the saturated and unsaturated FA parts in the FA chains and water. As a result, the individual T_2 values could be measured for these groups. We observed that the =CH–C**H**$_2$–CH= protons in all emulsions (i.e., protons close to unsaturated carbons) had much longer T_2 (263 to 478 ms) than did the –(C**H**$_2$)$_n$– protons (i.e., protons next to the saturated carbons) in the corresponding emulsions (T_2 values of 102 to 163 ms) (Table 1). This can be related to the molecular environments of the saturated and unsaturated parts of the FA chain within an oil droplet. The higher mobility (i.e., longer T_2) of the unsaturated parts, compared with the saturated parts, suggests that the unsaturated components tended to stay in the core of the oil droplets. This experimental result supports the hypothesis (7) that the more unsaturated FA are buried in the oil core of oil-in-water emulsions.

In WPI-based emulsions produced by the different processes, the T_2 values for the –(C**H**$_2$)$_n$– protons were shorter for microfluidized than for homogenized emulsions (Table 1). This might indicate an average slower mobility of the more saturated part of the FA chains on the oil droplet interface of microfluidized emulsions compared with those processed by homogenization. This may have resulted from the larger surface area of the microfludized WPI emulsions. Emulsion systems based on different surfactants (e.g., WPI- vs. lecithin-based emulsions) cannot be compared with each other in terms of T_2 values and molecular mobility, as there are additional physical factors that come into play, e.g., different molecular mobilities, emulsion interfacial structures, interactions between the oil and surfactant, and so on. Lecithin is oil-like and could interact more with tuna oil than does WIP. Therefore, the proton T_2 of the oil might include a contribution from additional oil–lecithin interactions. Consequently, T_2 measured from lecithin-based emulsions could not be used to interpret the surface interaction between the oil and surfactant as two separate molecular subsystems.

The longer T_2 of water molecules in the continuous phase of microfluidized, compared with homogenized, WPI-based emulsions (Table 1) suggested that there was less interaction of water with proteins in the continuous phase in microfluidized emulsions. This agreed with the fact that more proteins would be needed to cover the larger surface areas (smaller particle sizes) of the microfluidized WPI-based emulsions; therefore, less would be left in the continuous phase. The same trend was seen in the lecithin-based emulsions.

Correlations among T_2, particle size, and oxidative stability. T_2 values of the saturated parts of the FA chains were shorter for microfluidized WPI-based emulsions than for the corresponding homogenized emulsions. This indicated that oil molecules were affected more by interaction with surfactant molecules in the microfluidized WPI-based emulsions. They had more restricted motion, on average, indicating larger surface areas relative to volume. Therefore, the average particle sizes were smaller, which agreed with light-scattering measurements (Table 1). As mentioned, the T_2 of oil protons measured for lecithin-based emulsions could not be interpreted in the same way as those for WPI-based emulsions.

That lipid oxidation can be accelerated by increasing the interfacial area is well documented, but it can also be unaffected by a change in the surface area, depending on whether there are excesses or a limited amounts of oxidation reactants (2,7,8). In this study, for microfluidized WPI-based emulsions, the total particle surface area was about seven times that of the corresponding homogenized emulsion, whereas the amount of propanal formed by the microfluidized emulsions was only about 1.5 times that formed by homogenization during 8 wk of storage. The much shorter T_2 of saturated parts of the FA chains in TG for microfluidized WPI-based emulsions (Table 1) is related to a reduced mobility. If the motion of FA chains in smaller particles were more restricted, it would also slow down oxygen, free radical, and heavy metal pro-oxidant movements and so reduce the oxidation rate. Oxygen, free radical, and pro-oxidant movements are among the many factors affecting the oxidation of n-3 emulsions (8,9). Balinov (17) reported that molecules in discrete droplets had a much lower apparent dif-

fusion coefficient compared with molecules in the continuous phase, and that the value in the continuous phase was similar to the value in the corresponding bulk phase, which could support the NMR analysis results in this study.

The much longer T_2 of water molecules in the continuous phase of lecithin-based emulsions compared with those in WPI-based emulsions suggests that water in the continuous phase of the lecithin-based emulsions was less affected by the particle surface. Hence, molecules in this phase had more free motion, which would be a factor contributing to faster oxidation rates in the lecithin-based emulsions. This conclusion from our NMR analysis was also supported by the increased concentration of propanal for the lecithin-based emulsions.

ACKNOWLEDGMENTS

We are grateful to Food Science Australia, the CSIRO Food Futures Flagship, the CSIRO Emerging Science Program, and CSIRO Manufacturing and Infrastructure Technology for financial support. We thank Claudio Ceccato and Andrew Lawrence for technical assistance.

REFERENCES

1. de Deckere, E.A.M., O. Korver, P.M. Verschuren, and M.B. Kantan, Health Aspects of Fish and n-3 Polyunsaturated Fatty Acids from Plant and Marine Origin, *Eur. J. Clin. Nutr. 52*:749–753 (1998).
2. Trautwein, E.A., n-3 Fatty Acids—Physiological and Technical Aspects for Their Use in Food, *Eur. J. Lipid Sci. Technol. 103*:45–55 (2001).
3. Andersen, S., Omega-3 Fatty Acids in Food Fortification, *Leatherhead Food RA Food Ind. J. 1*:33–40 (1998).
4. Jacobsen, C., K. Hartvigsen, P. Lund, J. Adler-Nissen, G. Hølmer, and A.S. Meyer, Oxidation in Fish-Oil Enriched Mayonnaise, *Eur. Food. Res. Technol. 210*:242–257 (2000).
5. Augustin, M.A., and L. Sanguanri, Polyunsaturated Fatty Acids: Delivery, Innovation and Incorporation into Foods, *Food Aust. 55*:294–296 (2003).
6. Sharma, R., P. Sanguansri, R. Marsh, L. Sanguansri, and M.A. Augustin, Applications of Microencapsulated Omega-3 Fatty Acids in Dairy Products, *Aust. J Dairy Sci. Technol. 58*:203 (2003).
7. McClements, D.J., and E.A. Decker, Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems, *J. Food Sci. 65*:1270–1282 (2000).
8. Let, M.B., C. Jacobsen, E.N. Frankel, and A.S. Meyer, Oxidative Flavour Deterioration of Fish Oil Enriched Milk, *Eur. J. Lipid Sci. Technol. 105*:518–528 (2003).
9. Jacobsen, C., A.S. Meyer, and J. Adler-Nissen, Oxidation Mechanisms in Real Food Emulsions: Oil–Water Partition Coefficients of Selected Volatile Off-Flavour Compounds in Mayonnaise, *Z. Lewbensm. Unters. Forsch. A 208*:317–327 (1999).
10. Hu, M., D.J. McClements, and E.A. Decker, Emulsion Droplets Engineered to Improve the Oxidative Stability of n-3 Fatty Acids in Functional Foods, *Lipid Technol. 16*:79–82 (2004).
11. Osborn, H.T., and C.C. Akoh, Effect of Emulsifier Type, Droplet Size, and Oil Concentration on Lipid Oxidation in Structured Lipid-Based Oil-in-Water Emulsions, *Food Chem. 84*:451–456 (2004).
12. Courthaudon, J.L., E. Dickinson, and W.W. Christie, Competitive Adsorption of Lecithin and β-Casein in Oil in Water Emulsions, *J. Agric. Food Chem. 39*:1365–1368 (1991).
13. Donnelly, J.L., E.A. Decker, and D.J. McClements, Iron Catalysed Oxidation of Menhaden Oil as Affected by Emulsifiers, *J. Food Sci. 63*:997–1000 (1988).
14. Fomuso, L.B., M. Corredig, and C. Aloha, Effect of Emulsifier on Oxidation Properties of Fish Oil-Based Structured Lipid Emulsions, *J. Agric. Food Chem. 50*:2957–2961 (2002).
15. Hu, M., D.J. McClements, and E.A. Decker, Impact of Whey Protein Emulsifiers on the Oxidative Stability of Salmon Oil-in-Water Emulsions, *J. Agric. Food Chem. 51*:1435–1439 (2003).
16. Djordjevic, D., D.J. McClements, and E.A. Decker. Oxidative Stability of Whey Protein-Stabilized Oil-in-Water Emulsions at pH 3: Potential Omega-3 Fatty Acid Delivery System (Part B), *J. Food Sci. 69*:C356–C362 (2004).
17. Balinov, B., F. Mariette, and O. Söderman, NMR Studies of Emulsions with Particular Emphasis on Food Emulsions, in *Food Emulsions*, edited by S.E. Friberg, K. Larsson, and J. Sjobolm, Marcel Dekker, New York, 2004, pp. 593–632.
18. Falch, E., H.W. Anthonsen, D.E. Axelson, and M. Aursand, Correlation Between ^{1}H NMR and Traditional Methods for Determining Lipid Oxidation of Ethyl Docosahexaenoate, *J. Am. Oil Chem. Soc. 81*:1105–1110 (2004).
19. Dalgleish, D.G., S.M. Tosh, and S. West, Beyond Homogenization: The Formation of Very Small Emulsion Droplets During the Processing of Milk by a Microfluidizer, *Neth. Milk Dairy J. 50*:135–148 (1996).
20. Paquin, P., Technological Properties of High Pressure Homogenizers: The Effect of Fat Globules, Milk Proteins, and Polysaccharides, *Int. Dairy J. 9*:329–335 (1999).
21. Faraji, H., D.J. McClements, and E.A. Decker, Role of Continuous Phase Protein on the Oxidative Stability of Fish Oil-in-Water Emulsions, *J. Agric. Food Chem. 52*:4558–4564 (2004).
22. Meynier, A., V. Rampon, M. Dalgalarrondo, and C. Genot, Hexanal and *t*-2-Hexenal from Covalent Bonds with Whey Proteins and Sodium Caseinate in Aqueous Solution, *Int. Dairy J. 14*:681–690 (2004).
23. Weel, K.G.C., A.E.M. Boelruk, J.J. Burger, N.E. Claassen, H. Gruppen, A.G.J. Voragen, and G. Smith, Effect of Whey Protein on the *in vivo* Release of Aldehydes, *J. Agric. Food Chem. 51*:4746–4752 (2003).
24. Sacchi, R., I. Medina, L. Paolillo, One- and Two-Dimensional NMR Studies of Plasmalogens (alk-1-enyl-phosphatidylethanolamine), *Chem. Phys. Lipids 76*:201–209 (1995).
25. Mine, Y., K. Chiba, and M. Tada, Effect of the Oxidation of Free Fatty Acids on the Interfacial Absorptivity of Lysophosphatidylcholine/Free Fatty Acid/Ovalbumin Complexes, *Biosci. Biotechnol. Biochem. 56*:1814–1818 (1992).
26. Choi, S.G., and W.L. Kerr, Effects of Chemical Modification of Wheat Starch on Molecular Mobility as Studied by Pulsed ^{1}H NMR, *Lebensm. Wiss. Technol. 36*:105–112 (2003).

[Received February 9, 2005; accepted August 28, 2005]