Effects of Antioxidants and Humidity on the Oxidative Stability of Microencapsulated Fish Oil

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ABSTRACT: The effects of various antioxidants and RH on the oxidative stability of microencapsulated fish oil powder were investigated using PV and thiobarbituric acid tests. The microencapsulation process provided high encapsulation efficiency (≥88% of extractable fish oil). Without antioxidants, the encapsulated fat was 10 times more stable against oxidation than the surface fat, as determined by PV. α-Tocopherol, which is a lipophilic antioxidant, showed a greater antioxidative effect in both surface and encapsulated fats than ascorbyl palmitate, which is an amphiphilic antioxidant. According to TBARS values, the longest lag period was observed at 0% RH. Addition of >200 ppm α-tocopherol in a 10–30% RH range prolonged the oxidative stability of the microencapsulated fish oil powder.

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KEY WORDS: Antioxidants, fish oil, microencapsulation, oxidation, relative humidity.

Fish oil is in increasing demand in the food industry because it contains high levels of long-chain PUFA (LCPUFA) with important physiological functions and health benefits (1,2). However, relative to other edible oils, fish oil is highly susceptible to oxidation, mainly because of the high number of 1,4-pentadiene systems in its LCPUFA and the lack of endogenous antioxidants.

The oxidative deterioration of LCPUFA results in a loss of nutritional value and the development of off flavors (3). The usual approaches to minimizing oxidation are the addition of antioxidants and microencapsulation (4,5). Typically, the oil is homogenized with an emulsifier in water, and the resultant mixture is dried rapidly, most often in a spray dryer, to yield a powdered encapsulated product. Microencapsulation entraps sensitive materials such as LCPUFA in a wall matrix, and this wall serves as an oxygen and moisture barrier, resulting in increased oxidative stability (6–8).

Use of antioxidants in combination with suitable emulsifying systems (i.e., emulsifier, functional protein, and carbohydrate) are considered important protective factors against oxidation, but their mode of action in a dry state is not clearly understood.

It is certain that water plays a critical role in the lipid oxidation of foods. In general, the lipid oxidation of foods has been said to slow down in a low-moisture environment (8) but numerous studies have suggested that this generalized view does not apply to a number of systems (9). Extremely dry conditions have been reported to accelerate lipid oxidation in many foods (9–11). Based on hexanal and heptanal evolution, oxidation has been postulated to decrease as water activity (a_w) is increased from a completely dry state to the "monolayer" region (0.2–0.3 a_w) (9). Similar trends were also found in freeze-dried chicken breast myofibrils (10) and freeze-dried beef (11). However, contradicting reports also exist. In encapsulated form, orange oil and methyl linoleate have been found to be more oxidatively stable in very dry (0 a_w) and very moist (>0.75 a_w) states, whereas more rapid oxidation was found in the intermediate range (12–14). It is highly possible that the antioxidative or prooxidative roles of water may depend on the structure, composition, and microscopic heterogeneity of the food system.

In the present study we investigated the effects of antioxidants and RH on the oxidative stability of spray-dried microencapsulated fish oil during storage.

MATERIALS AND METHODS

Materials. Fish oil (refined menhaden oil, OmegaPure™) without antioxidant was kindly donated by Omega Protein, Inc. (Hammond, LA). Ascorbyl palmitate was purchased from ICN Biomedicals, Inc. (Costa Mesa, CA), α-tocopherol from Sigma-Aldrich (St. Louis, MO), potassium phosphate from Fisher Scientific (Pittsburgh, PA), and corn syrup solids (DE-36) from Roquette America (Keokuk, IA). Lecithin (BEAKIN LV3, PG-352) was obtained from Archer Daniels Midland (Decatur, IL), sodium caseinate from New Zealand Milk Products Inc. (Santa Rosa, CA), and amorphous fumed silica (CAB-O-SIL®) from Cabot Co. (Tuscola, IL).

Sample preparation. Microencapsulated fish oil powders were prepared as previously described for milk fat (15). The same formulation was used with fish oil (rather than milk fat). A coarse emulsion of 30% solid content was mixed, then homogenized in conditions known to achieve about 0.50 + 0.05 μm in oil droplet size as measured using a light-scattering machine (Coulter LS 230; Coulter Corporation, Miami, FL). The emulsion was pasteurized at 65°C for 30 min and then spray-dried at

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210°C inlet and 95°C outlet temperatures. The powders were vacuum-packaged in hermetically sealed pouches and stored at –40°C prior to the stability test.

Four kinds of fish oil powders were prepared: (i) control (without antioxidant), (ii) with 250 ppm α-tocopherol, (iii) with 250 ppm ascorbyl palmitate, and (iv) with 1000 ppm α-tocopherol. In the case of samples with antioxidants, α-tocopherol (250 or 1000 ppm/total fish oil) and/or ascorbyl palmitate (250 ppm/total fish oil) was dissolved in 3 mL of a hexane/isopropanol (3:1, vol/vol) mixture and added to the fish oil. The fish oil was then slowly stirred under nitrogen gas flow for 30 min to retard oxidation and to evaporate the solvent. The four emulsions were then prepared as described above.

The moisture content of the spray-dried powder was 1.1 ± 0.1% (g water/100 g powder × 100) in all samples, and the final composition (dry basis) of the microencapsulated fish oil powders was as follows: fish oil 40%, corn syrup solids (DE-36) 49.65%, sodium caseinate 7.5%, lecithin 2%, and potassium phosphate (dibasic) 0.85%. Two separate batches of fish oil powders were prepared in each case.

Storage. The fish oil powder (10 g) was uniformly spread into a disposable Petri dish (95 × 15 mm; Fisher Scientific) and the dish was put into a desiccator with a RH of 0, 11, 33, or 43%, prepared using silica gel and saturated LiCl, $MgCl_2$, and K_2CO_3 solutions (17). Two desiccators were used to duplicate each RH condition. All desiccators were then put into an incubator at 30°C. Samples were withdrawn at frequent time intervals for analyses.

Moisture content. Duplicate samples of approximately 2 g of powder were placed in an aluminum dish and dried for 24 h at 70°C and 29 in. Hg in a vacuum oven (Fisher Scientific, Fairlawn, NJ). Moisture content was calculated from the weight difference (AACC Method 44-01) (17).

Extraction of surface and encapsulated fats. Surface fat was extracted from 2.5 g of powder by adding 15 mL of hexane and vortexing for 2 min at room temperature, followed by decanting and drying under a nitrogen gas flow (18). Encapsulated fat was extracted by dispersing the washed powder (0.25 g of powder washed with 2 mL of hexane) in distilled water and extracting the fat with a hexane/isopropanol (3:1, vol/vol) mixture (18,19). For encapsulated fat, extraction procedures were repeated three times to increase the extraction efficiency. After three extractions, the collected clear organic phase was dried under a gentle nitrogen gas flow and the lipid content was determined gravimetrically.

PV. PV were monitored in both surface and encapsulated fractions during storage. The colorimetric method described by International IDF Standards (20) was used with some modifications, as described by Shantha and Decker (21) and Hardas *et al.* (18). Samples (0.01–0.10 g fat) were mixed in a disposable glass tube with 3 mL of chloroform/methanol (2:1, vol/vol). A solution of 25 µL of ammonium thiocyanate and a second solution of Fe^{2+} (0.005 g/mL) were added. The final mixture was vortexed briefly and incubated for 20 min in a dark room. After incubation, the absorbance was measured using a UV/vis spectrophotometer (Lambda 3; PerkinElmer, Wilton, CT) at 500 nm. PV was quantified on the basis of a standard curve prepared from cumene hydroperoxide.

TBARS. TBARS values of microencapsulated fish oil powders were determined using a modified method of Srinivasan and Xiong (22) by mixing fish oil powder (0.1 g) with 2.0 mL of a 7.5% TCA/0.1% propyl gallate/100 µM diethylenetriaminepenta-acetic acid solution using a vortex mixer (Vortexer 2; VWR Scientific Products, Boston, MA). The mixture was then centrifuged at 1100 × *g* for 5 min. A 1-mL aliquot of the supernatant was then mixed with 1 mL of 0.02 M thiobarbituric acid (TBA) solution. The TBA sample solutions were incubated for 15 min in a boiling water bath, centrifuged (5 min at 1100 × *g*), read spectrophotometrically at 532 nm using a UV/vis spectrophotometer (Lambda 3; PerkinElmer), and quantified based on the molar extinction coefficient of malonaldehyde (1.56×10^5 M^{-1} cm^{-1}) (22).

Sensory evaluation. Sensory evaluation of the microencapsulated fish oil powders was performed using a ranking test (23). Ten judges were selected and trained using standard samples made from varying mixtures of fresh corn oil and extremely oxidized (21 d) fish oil powder (with 0 ppm antioxidants). Eight samples (0 and 14 d with 0 ppm antioxidants, with 250 ppm ascorbyl palmitate, with 250 ppm α-tocopherol, and with 1000 ppm α-tocopherol) were used. After each sensory test, panelists ranked the sample from 1 (no rancid or fishy odor) to 9 (extremely rancid or fishy odor).

Statistical analysis. Extractability, PV, and TBARS values were analyzed from two replicate powders. All extractability and PV measurements were performed twice, and all TBARS measurements were performed four times. All statistically significant tests were further analyzed by the Duncan test (SAS software, version 6.12; SAS Institute, Inc., Cary, NC) at a 95% confidence level.

RESULTS

Effect of processing on the oxidation of fish oil. Figure 1 shows the changes in PV of the fish oil emulsion during processing. The PV of the fish oil emulsion gradually increased from 9.5 ± 0.4 mmol/kg fat in fresh bulk fish oil to 17.4 ± 4.0 mmol/kg fat in the spray-dried powder. During processing, fish oil is exposed to air, high pressure, and high temperature, which leads to an increase in lipid oxidation.

Extraction of surface and encapsulated fats. Figure 2 shows the amounts of extracted surface and encapsulated fats obtained from the microencapsulated fish oil powder during storage (30°C and 11% RH). The amounts of extractable surface fat decreased gradually but only slightly over time. This decrease may due to oxidation-induced polymerization, which reduces extractability of the lipid material. Hardas and coworkers (18,19) found the same phenomenon in encapsulated milk powder. More than 88% of total fat was extracted as encapsulated fat during storage in all samples, with no significant change during storage.

PV. Figures 3A and 3B show PV for surface and encapsulated fish oils extracted from the microencapsulated powder

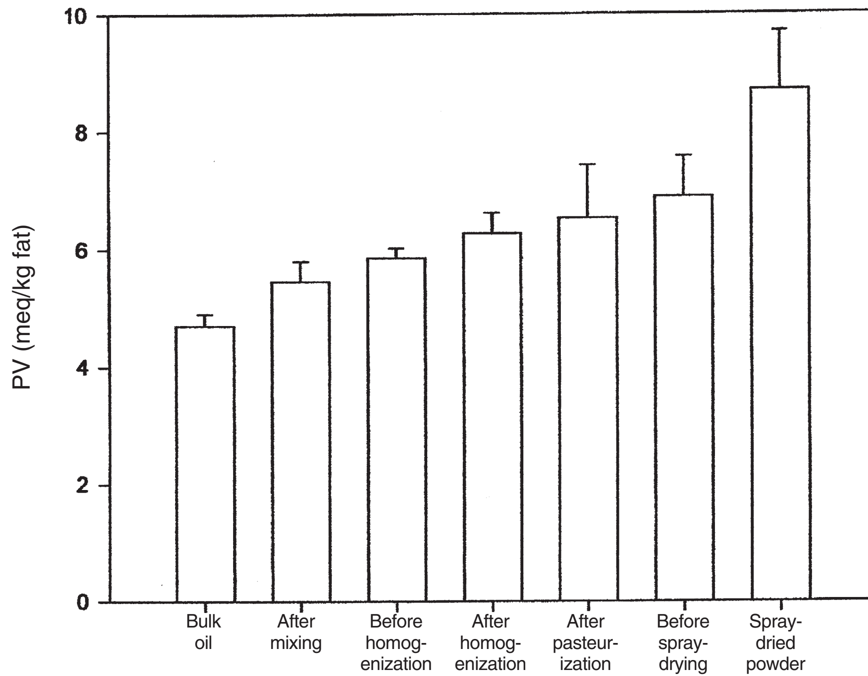

FIG. 1. Effects of processing steps on the PV of the fish oil emulsion (control). Error bars represent SD.

upon storage at 30°C and 11% RH. Both the surface and encapsulated fractions exhibited similar PV profiles in corresponding antioxidant treatments, but the surface oil exhibited a 10-fold greater PV than the encapsulated oil. The control and 250-ppm ascorbyl palmitate treatments both increased in PV rapidly (within a few days), whereas the α-tocopherol-treated samples showed only a slight increase in PV during the same period. The data indicated that α-tocopherol effectively

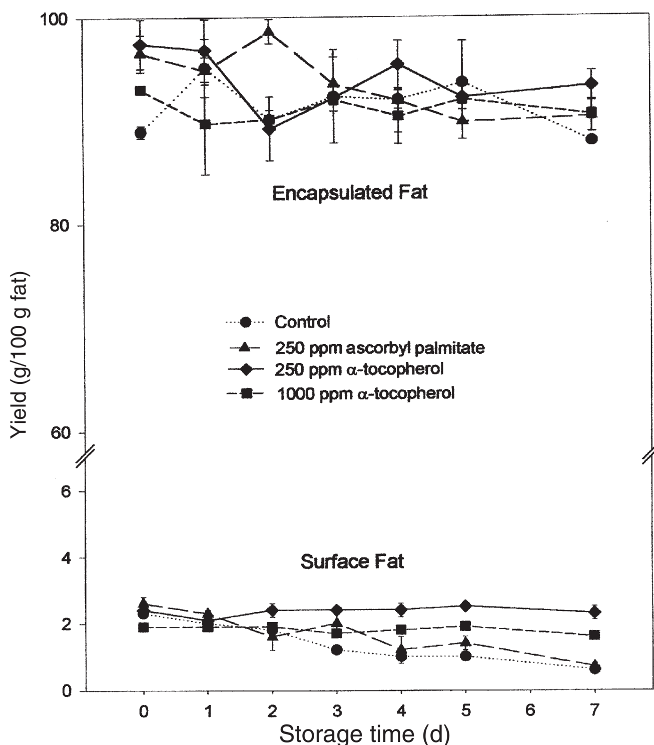

FIG. 2. Extraction yields for surface and encapsulated fish oil from microencapsulated powder during storage at 30°C and 11% RH. Error bars represent SD.

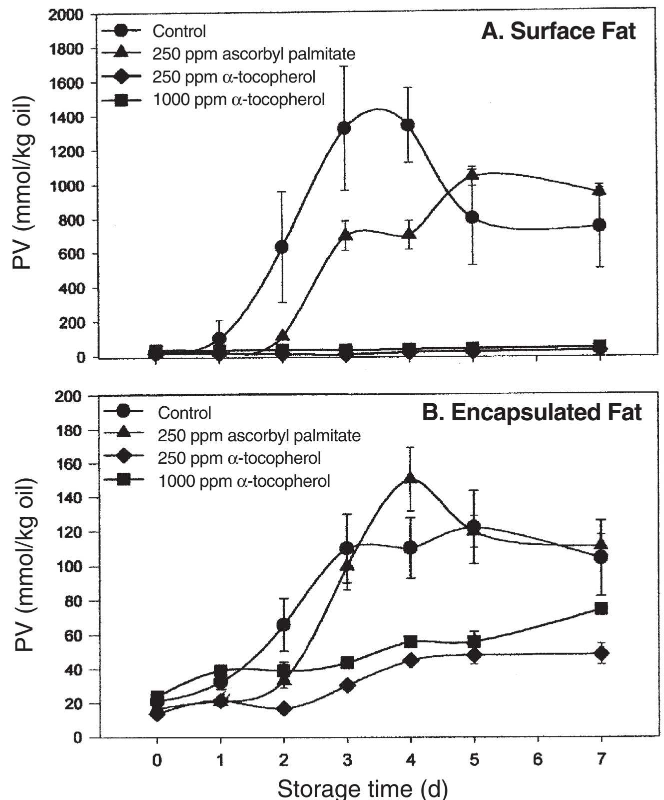

FIG. 3. Change in the PV of fish oil from microencapsulated fish oil powder during storage at 30°C and 11% RH. Error bars represent SD.

delayed the oxidation process both on the inside and on the surface of the powder.

Sensory evaluation. Sensory evaluation was performed using a ranking test (23). All 0-d powders ranked in the range of 3.8–5.5, with the 1000-ppm α-tocopherol sample performing the best, although it was not significantly different from other antioxidant-treated samples ($P \leq 0.05$) (Fig. 4). In aged (14 d at 11% RH, 30°C) samples, all samples were ranked higher than 5. Only the 1000-ppm α-tocopherol treatment resulted in a significantly less rancid odor after 14 d of storage, ranking 5.8 (Fig. 4).

Effect of RH. One of the most important factors that determines the stability of dried powders is the presence of moisture. TBARS values were used to investigate the oxidative stability of powders after storage in various humidity environments. Figure 5 depicts TBARS development during storage at 0, 11, 33 and 43% RH in the control and α-tocopherol-treated samples.

In the control, TBARS values at all RH showed an extended lag period, followed by a dramatic increase and then a gradual decrease, leveling off at some asymptotic value (depending on RH, Fig. 5). Increasing the RH slightly increased the lag time of the control samples. However, there appeared to be no major difference in lag times and peak TBARS values for samples stored at various RH (Fig. 5). Only a slight delay in lag time in the control (from 6–7 d to 10 d) was observed as the storage RH increased to 33% and then to 43% (Fig. 5). The peak TBARS value decreased slightly when going from 0 to 11% RH and then increased with a further increase in RH to 43% (Fig. 5). These peak TBARS values, however, fell within the 100–155 mM/kg powder range, corresponding to approximately a 30% variation.

The TBARS values of 250- and 1000-ppm α-tocopherol-treated samples showed a much longer lag period than the control among all the RH studied (Fig. 5). The peak values were not only delayed but also lowered compared with those of the control samples, suggesting reduced oxidative products. For 1000 ppm α-tocopherol (0 and 11% RH), the sample appeared more stable over an extended time and only increased slightly after 14 d (0% RH) and 22 d (11% RH), respectively (Figs. 5A and 5B).

In all samples, the highest peak and asymptotic TBARS values were observed at 0% RH. These tended to decrease slightly at 11% RH and then increase at 33 and 43% RH (Fig. 5). The lowered TBARS value at 11% RH was not very large (from 100 to 120 mM/kg powder in the control—a decrease of approximately 20% from 0% RH).

TBARS values, as a function of RH, changed dramatically with storage time. For instance, after 9 d of storage, the control showed the highest TBARS value at 0% RH, whereas after 14 d the highest TBARS value was at 43% and the lowest was at 11% RH. In the presence of α-tocopherol, the TBARS value remained <20 mM/kg powder, but a slightly lower TBARS value was observed at 11% RH. Hence, storage of the powder with α-tocopherol was the best at an intermediate RH (11–33%).

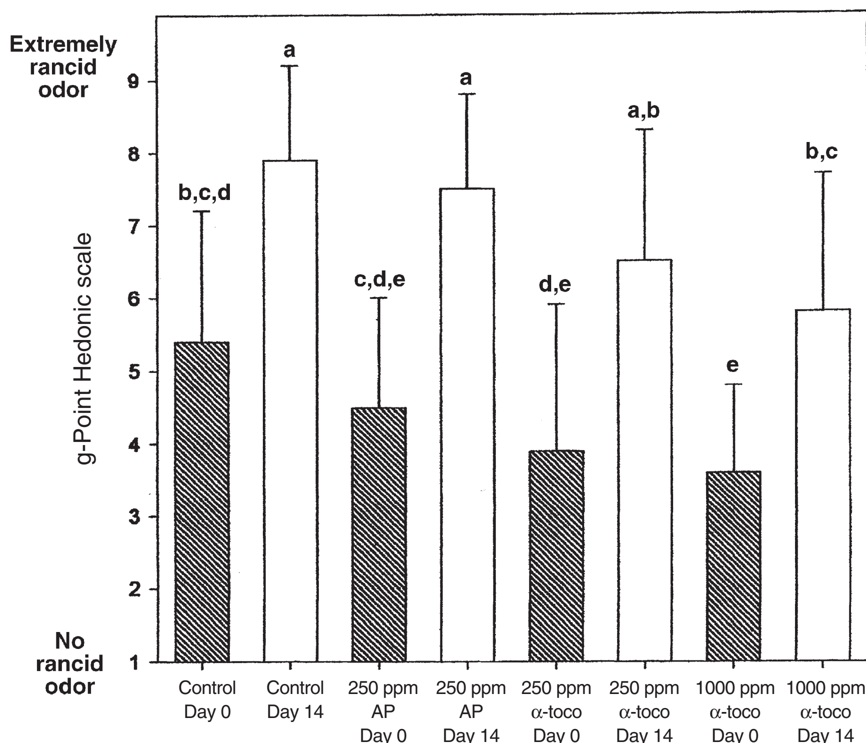

FIG. 4. Sensory evaluation of microencapsulated fish oil powder with and without various antioxidants. Different letters above the bar represent that values are significantly different at a level of $P \leq 0.05$. Error bars represent SD. AP, ascorbyl palmitate; α-toco, α-tocopherol.

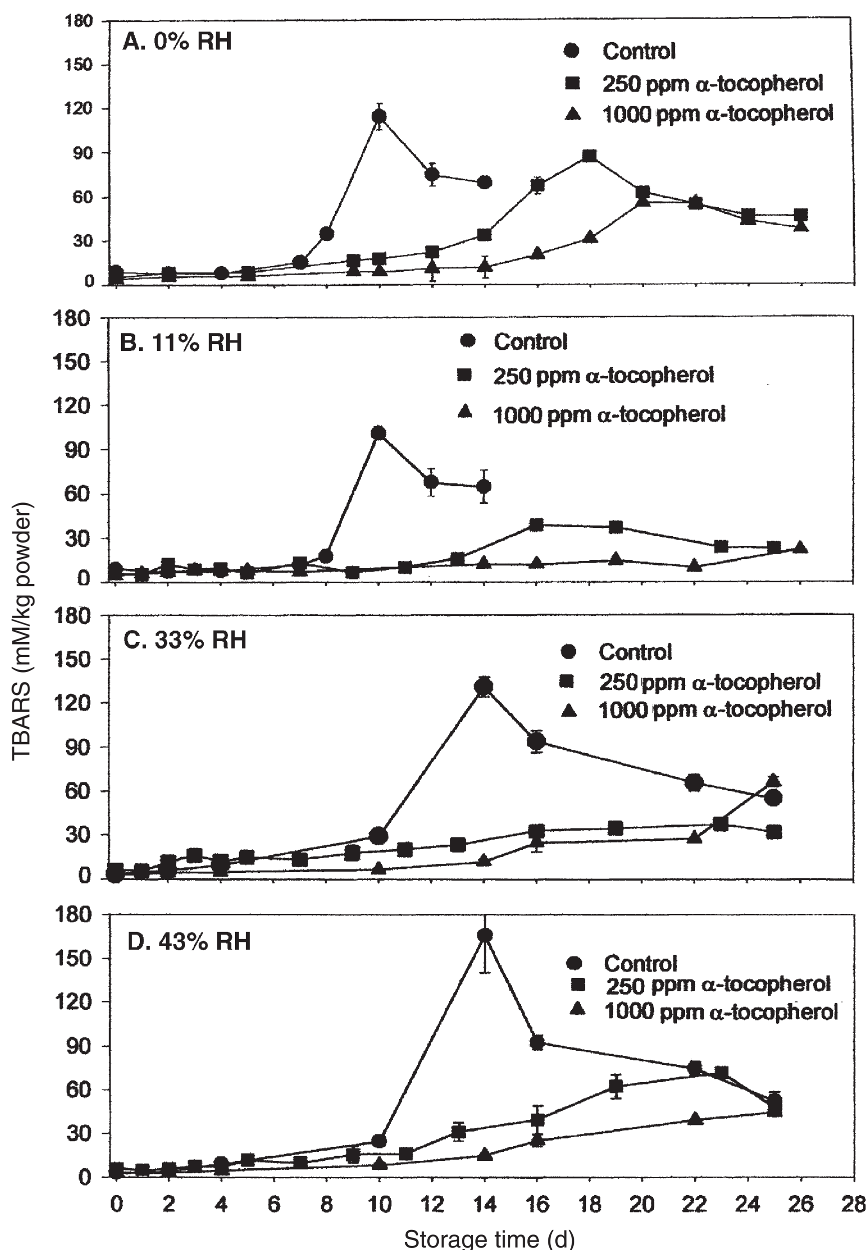

FIG. 5. Effect of RH on the TBARS of microencapsulated fish oil powder during storage at 30°C. Error bars represent SD.

On the other hand, an extension in the lag time may be indicative of the product's shelf-life. In such a case, based on lag-time observations, no dramatic effect of RH on the lag time and shelf-life stability of the encapsulated fish oil powders was found. α-Tocopherol treatments at >200 ppm effectively prolonged the fish oil product shelf-life relatively independently of the RH.

This work demonstrates that oxidation of lipids in microencapsulated fish oil powders is largely influenced by an interplay of chemical and physical factors in the dry state. The effects of specific antioxidants and environmental factors are under an ongoing investigation to improve the product's shelf life and enhance its industrial applications.

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REFERENCES

1. Newton, I.S., Food Enrichment with Long-Chain n-3 PUFA, *INFORM 7*:169–177 (1996).
2. Haumann, B.F., Nutritional Aspects of n-3 Fatty Acids, *INFORM 8*:428–447 (1997).
3. Velasco, J., M.C. Dobarganes, and G. Márquez-Ruiz, Oxidación de fracciones de aceite libres y encapsulades en aceites de pescado microencapsulados, *Grasas Aceites 51*:439–446 (2000).

4. Driezak, J.D., Microencapsulation and Encapsulated Ingredients, *Food Technol. 42*:136–151 (April 1988).
5. Matsuno, R., and S. Adachi, Lipid Encapsulation Technology—Techniques and Applications to Food, *Trends Food Sci. Technol. 4*:256–261 (1993).
6. To, E.C., and J.M. Flink, Collapse, a Structure Transition in Freeze-Dried Carbohydrates. 2. Effect of Solute Composition, *J. Food Technol. 13*:567–581 (1978).
7. Karel, M., M.P. Buera, and Y. Roos, Effects of Glass Transitions on Processing and Storage, in *The Glassy State in Foods*, edited by J.M.V. Blanshard amd P.J. Lillford, Nottingham University Press, Loughborough, Leicestershire, United Kingdom, 1992.
8. Maloney, J.F., T.P. Labuza, D.H. Wallace, and M. Karel, Autoxidation of Methyl Linoleate in Freeze-Dried Model Systems. I. Effect of Water on the Autocatalyzed Oxidation, *J. Food Sci. 31*:878–896 (1966).
9. Labuza, T.P., H. Tsuyuki, and M. Karel, Kinetics of Linoleate Oxidation in Model Systems, *J. Am. Oil Chem. Soc. 46*:409–416 (1969).
10. Smith, D.M., S.H. Noormarji, J.F. Price, M.R. Bennink, and T.J. Herald, Effect of Lipid Oxidation on the Functional and Nutritional Properties of Washed Chicken Myofibrils Stored at Different Water Activities, *J. Agric. Food Chem. 38*:1307–1312 (1990).
11. Quaglia, G.B., F. Sinesio, D. Veccia-Scavalli, V. Avalle, and G. Scalfati, Effect of Water Activity on Oxidative Deterioration of Freeze-Dried Beef, *Int. J. Food Sci. Technol. 23*:241–246 (1988).
12. Nelson, K.A., and T.P. Labuza, *Lipid Oxidation in Food*, edited by A.J. St. Angelo, ACS Symposium Series No. 500, American Chemical Society, Washington, DC, 1992, pp. 93–103.
13. Ma, Y., G.A. Reineccius, T.P. Labuza, and K.A. Nelson, The Stability of Spray-Dried Microcapsules as a Function of Glass Transition Temperature, paper presented at the Institute of Food Technologists Annual Meeting, New Orleans, LA, June 17–18, 1992.
14. Kahl, J.L., W.E. Arta, and E.G. Schanus, Effects of Relative Humidity on Lipid Oxidation in a Model System, *Lipids 23*:275–279 (1988).
15. Buma, T.J., Free Fat in Spray-Dried Whole Milk. 2. Evaluation of Methods for Determination of Free-Fat Content, *Neth. Milk Dairy J. 25*:42–52 (1971).
16. Greenspan, L., Humidity Fixed Points of Binary Saturated Aqucous Solutions, *J. Res. Nat. Bur. Stand. A 81*:89–96 (1977).
17. American Association of Cereal Chemists, *Approved Methods of the AACC*, 10th edn., American Association of Cereal Chemists, St. Paul, MN, 2000, Method 44-01.
18. Hardas, N., S. Danviriyakul, J.L. Foley, W.W. Nawar, and P. Chinachoti, Effect of Relative Humidity on the Oxidative and Physical Stability of Encapsulated Milk Fat, *J. Am. Oil Chem. Soc. 79*:151–158 (2002).
19. Hardas, N., S. Danviryakul, J.L. Foley, W.W. Nawar, and P. Chinachoti, *Lebensm. Wiss. Technol. 33*:506–513 (2000).
20. International IDF Standards, International Dairy Federation, Brussels, Belgium, 1991, Sec. 74A.
21. Shantha, N.C., and E.A. Decker, Rapid, Sensitive, Iron-Based Spectrophotometric Methods for Determination of Peroxide Values of Food Lipids, *J. Assoc. Off. Anal. Chem. Int. 77*:421–424 (1994).
22. Srinivasan, S., and Y.L. Xiong, Sodium Chloride-Mediated Lipid Oxidation in Beef Heart Surimi-like Material, *J. Agric. Food Chem. 44*:1697–1703 (1996).
23. Poste, L.M., D.A. Mackie, G. Butler, and E. Larmond, in *Laboratory Methods for Sensory Analysis of Food*, edited by E. Larmond, Canada Communication Group–Publishing Centre, Ottawa, Canada, 1991, pp. 64–67.

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