# Edible Coatings on Frozen King Salmon: Effect of Whey Protein Isolate and Acetylated Monoglycerides on Moisture Loss and Lipid Oxidation

YVONNE M. STUCHELL and JOHN M. KROCHTA

──────────── ABSTRACT ────────────

Whey protein isolate (WPI) and acetylated monoglyceride (AMG) coatings were evaluated for effectiveness against moisture loss and lipid oxidation of frozen King salmon. A model gel material was also used to screen coatings for effectiveness against moisture loss. Coatings of low-melting-point AMG used alone or after applying WPI solution or WPI powder were effective in reducing the rate of moisture loss by 42–65% during the first 3 wk of storage. Onset of lipid oxidation was delayed and peak peroxide values were reduced in samples coated with WPI solution/antioxidant overspray or those containing low-melting-point AMGs. No differences in effectiveness were found among the coating treatments.

Key Words: salmon, edible films, whey protein, WPI, lipid oxidation, moisture loss

## INTRODUCTION

FROZEN STORAGE OF FISH is an effective means of preservation which prevents or decreases undesirable chemical changes while maintaining many characteristics of fresh fish. However, during long-term storage, fish and fish products may still deteriorate at a much reduced rate. Edible, protective film coatings can lessen or prevent quality changes and prolong storage life in foods such as frozen fish by acting as barriers to control moisture transfer, oxygen uptake and loss of volatile aromas and flavors. Such coatings must have appropriate mechanical and sensory properties. Edible films can be made from proteins, polysaccharides, lipids or a combination of these materials as either bilayers or emulsions (Guilbert, 1986; Kester and Fennema, 1986; Krochta, 1992).

Many types of coating materials have been tested in attempts to maintain quality in frozen foods. Ice glazing has often been used to retard lipid oxidation and moisture loss (Khayat and Schwall, 1983), but brittleness of ice and loss due to sublimation requires that fish be reglazed periodically during frozen storage (Wheaton and Lawson, 1985). Addition of corn syrup solids, cellulose gums and pectinates to glazing solutions has had limited success and acceptance (Ijichi, 1978). Gelling polysaccharides, such as carrageenan and alginate, have been applied as coatings to frozen foods, but were no more effective than ice glazing (Ijichi, 1978; Shaw and Secrist, 1977). Lipid coatings such as acetylated monoglycerides (AMGs) are most frequently used and are expected to provide a good moisture barrier because of their hydrophobicity. Chicken breasts coated with AMG (Myvacet 7-15) and stored at −18°C were comparable after cooking to controls wrapped in polyvinylidene chloride film (Zabik and Dawson, 1963). Hirasa (1991) used AMG (Myvacet 5-07) to coat Silver salmon pieces which were then stored at −10°C for 10 wk, and noted a decrease in relative moisture content of only 2.7% during that time. This coating, however, formed visible cracks during freezing and moisture loss probably occurred through these flaws.

Proteins have not been investigated as extensively as other biopolymers for edible coatings and films (Krochta, 1992). A 3% aqueous gelatin coating containing antioxidants at 0.15% total concentration provided excellent protection against rancidification in cut poultry when compared to uncoated controls and gelatin coating alone (Klose et al., 1952). Films from whey protein isolate (WPI) have been shown to be excellent oxygen barriers (McHugh and Krochta, 1994). Casein and AMG (Myvacet 5-07) emulsion coatings were used on frozen Silver salmon pieces which were stored at −10°C, and reduced moisture loss when compared to uncoated salmon (Hirasa, 1991). However, the characteristics of multicomponent films have not been fully evaluated at frozen temperatures.

Whey protein has not been tested as a coating under frozen conditions. Previous work with AMG coatings has been with those which are solid at room temperature (≈23°C) and thus they cracked at freezing temperatures. We studied AMGs which are liquid at room temperature and exhibited no visible cracking under freezing conditions. Coatings explored previously for frozen fish were quite thick and opaque and were designed to be easily removed prior to fish analysis after frozen storage (Hirasa, 1991). However, coatings we used were thin, transparent to translucent, and could not be removed readily from the samples. Our objective was to evaluate the effectiveness of various WPI and AMG coatings and compare their effects on rates of moisture loss and lipid oxidation in frozen King salmon.

## MATERIALS & METHODS

### Materials

Fresh farmed King salmon (*Oncorhynchus tshawytscha*) were purchased from Pacific Fresh Seafood Company (West Sacramento, CA). BiPRO whey protein isolate (WPI) was supplied by Le Sueur Isolates (Le Sueur, MN). Acetylated monoglycerides (AMG) Myvacet 9-08® and Myvacet 9-45®, were obtained from Eastman Chemical Products Inc. (Kingsport, TN). Myvacet 9-08 is a hydrogenated coconut oil, 96% acetylated, with an iodine value of 2 and melting point range of −12 to −14°C. Myvacet 9-45 is a partially hydrogenated soybean oil, 96% acetylated, with an iodine value of 43–53 and melting point range 4–12°C. Kelgum®, a xanthan gum/locust bean gum mixture, was supplied by Kelco (San Diego, CA); and Firm-Tex® starch was supplied by National Starch and Chemical Company (Bridgewater, NJ). L-ascorbic acid, citric acid monohydrate, chloroform, methanol, benzene, and hydrochloric acid were purchased from Fisher Scientific Co. (Pittsburgh, PA). Ammonium thiocyanate, ferrous sulfate heptahydrate and ferric chloride hexahydrate were purchased from Aldrich Chemical Company Inc. (Milwaukee, WI). Barium chloride was obtained from Sigma Chemical Co. (St. Louis, MO). All chemicals used were ACS grade.

### Treatments

Ten treatments were tested: Uncoated (control); 10% WPI solution spray; 10% WPI solution spray followed by 5% antioxidant overspray; Myvacet 9-08 AMG spray; Myvacet 9-45 AMG spray; 10% WPI solution spray followed by Myvacet 9-08 overspray; WPI powder dusting followed by Myvacet 9-08 overspray; WPI powder dusting followed by Myvacet 9-45 overspray; 10% WPI:10% Myvacet 9-08 emulsion spray; 10% WPI:10% Myvacet 9-45 emulsion spray.

**Table 1—Average weight of coatings applied to fish samples[a]**

| Treatment | Wt spray or powder coating (g) | Wt overspray coating (g) |
|---|---|---|
| Uncoated | — | — |
| 10% WPI spray | 1.61 ± 0.46 | — |
| 10% WPI spray/Antioxidant overspray | 1.27 ± 0.20 | 0.76 ± 0.17 |
| 10% WPI: 10% Myv 9-08 emulsion spray | 3.21 ± 0.48 | — |
| 10% WPI: 10% Myv 9-45 emulsion spray | 2.45 ± 0.26 | — |
| 10% WPI spray/Myv 9-08 overspray | 1.27 ± 0.18 | 0.58 ± 0.12 |
| WPI Powder/Myv 9-08 overspray | 0.47 ± 0.07 | 0.99 ± 0.22 |
| WPI Powder/Myv 9-45 overspray | 0.45 ± 0.06 | 0.41 ± 0.21 |
| Myv 9-08 spray | 1.00 ± 0.31 | — |
| Myv 9-45 spray | 1.78 ± 0.21 | — |

[a] Average weight of fish samples to which coating was applied, 48g; average exposed surface area, 63 cm[2].

## Coating preparation

A 10% (w/w) WPI solution was prepared using 300 g WPI powder and 2700g distilled water, then heating for 20 min in a 90°C water bath and cooling to 5°C with stirring. Both 10% WPI:10% Myvacet 9-08 and 10% WPI:10% Myvacet 9-45 emulsion solutions were made by adding 100g WPI powder to 800 g distilled water, then heating and cooling as with WPI solution. Next, 100g of either Myvacet 9-08 or Myvacet 9-45 was added, and the solution was emulsified for 3 min at 13500 rpm followed by 8 min at 20500 rpm using an Ultra-Turrax T-25 homogenizer with an 10 mm KG probe. The solution was cooled to 5°C prior to application. A 5% antioxidant solution composed of 2.5% each citric and ascorbic acid (w/w in distilled water) was made and cooled to 5°C prior to application.

## Preparation of model gel for preliminary tests

A model gel-like material was developed for evaluating effectiveness of frozen coatings in reducing moisture loss. A 1% Kelgum/2% Firm-Tex (w/w in distilled water) solution was made using a Braun (type 4-172) handheld mixer. The solution was heated with stirring to 78°C and held at that temperature for 1 min. Round plastic petri dishes 35 × 10 mm were filled with hot solution and the cover placed on the dish to remove excess material. The dishes were set on a level surface to cool and gel at ambient temperature, then stored at 4°C until coating. At the time of coating, gels were removed from the dishes. Coating was accomplished by dipping each gel into the coating material, allowing excess to drip off, placing the coated gel into a 8 cm hexagonal weighing dish, and freezing at −23°C. Triplicate samples of each coating were tested.

## Fish sample preparation

Twenty King salmon (*Oncorhynchus tshawytscha*), 2.75–3.65 kg whole gutted fish, were purchased 3–4 days post slaughter, filleted, skinned, wrapped in plastic, packed in crushed ice and stored at 2°C overnight. Fish fillets were partially frozen prior to trimming to remove outer flesh containing dark muscle, resulting in a sample thickness of 1.25–2.00 cm. Samples (6.3 cm diameter, avg wt 48g) with average exposed surface area (top and side surface) of 63 cm² were frozen at −28°C. The WPI powder coating was applied to samples prior to freezing to ensure good adhesion. Protein solution and emulsion coatings were applied as a fine spray after fish freezing using a home garden sprayer. Myvacet and antioxidant oversprays were applied using Crown® Spra-Tool (Fisher Scientific Co., Pittsburgh, PA). Time between fish purchase and sample coating was <38 hr. After coating, samples were stored at −23°C. Table 1 gives the average weight of each spray or powder coating and overspray applied to the fish samples. Dishes of pure ice, AMG coated ice, and AMG were also placed in the freezer as references.

## Moisture loss

The total weight change of each sample from time of coating to date of sampling was used to assess weight loss of stored samples. At each sampling time, samples were visually examined for cracks in the coating. Moisture content of each sample was reported as relative moisture content and represents the average of five measurements for each treatment. For comparison among treatments, the average rate of moisture loss over the initial 3-wk period and the 3-thru-8-wk period were calculated.
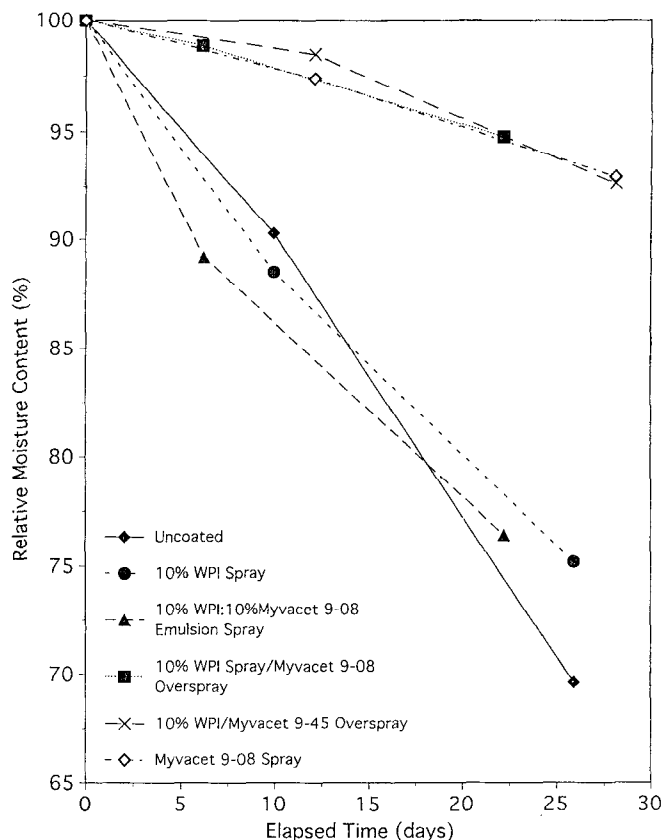


**Fig. 1—Relative moisture content of frozen model gels coated with WPI and acetylated monoglyceride edible coatings and stored at −23°C.**

## Lipid extraction

Lipid was extracted from the fish sample using the method of Bligh and Dyer (1959) with some modifications. About 1/4 of the fish sample with its coating was chopped into small pieces with a sharp knife and placed into a 125 mL high density polyethylene flask. The flask was placed in an ice bowl, 10 mL chloroform and 20 mL methanol were added, and the mixture was homogenized for 2 min at 13500 rpm with an Ultra-Turrax T-25 homogenizer using an 8 mm Kg probe. Next, 10 mL chloroform was added and homogenization was continued for an additional 30 sec. Finally, 10 mL distilled water was added and the mixture was homogenized for 30 sec more. This mixture was divided into two 50 mL centrifuge tubes and centrifuged 30 min at 1500×g using an IEC centrifuge. The chloroform layer was removed and stored at −28°C until testing.

## Determination of lipid oxidation

Lipid oxidation was determined using the method of Chapman and Mackay (1949) to determine peroxide value of the sample. The lipid/chloroform mixture was evaporated at 50°C in a water bath with nitrogen blowing over the solution to avoid further oxidation. Aliquots of lipid were weighed accurately, mixed with 9.9 mL benzene:methanol (7:3), and vortexed until completely dissolved. One drop each of ammonium thiocyanate and ferrous chloride solution were added, vortexed, heated at 50°C in a water bath for 2 min, and then cooled quickly to ambient temperature. The absorbance of each solution was measured at 510 nm using a Shimadzu UV-1201 UV-Vis spectrophotometer and used to calculate the peroxide value. Peroxide values represent the average of duplicate measurements for each sample and 5 samples for each treatment.

## Statistical analysis

StatView 4.0 was used for all statistical analyses (Abacus Concepts, Berkeley, CA). Analyses of variance and Fisher PLSD multiple comparisons were performed.

Table 2—Average rate of moisture loss as affected by thickness of coatings applied to fish samples

| Treatment | Avg total thickness of coatings[a] (mm) | Average rate of moisture loss | | | |
|---|---|---|---|---|---|
| | | 0–3 wk (g/cm² wk) | per mm coating, 0–3 wk (g mm/cm² wk) | 3–8 wk (g/cm² wk) | per mm coating, 3–8 wk (g mm/cm² wk) |
| Uncoated | — | 0.019 | — | 0.015 | — |
| 10% WPI spray | 1.7 | 0.025 | 0.043 | 0.017 | 0.029 |
| 10% WPI:10% Myv 9-08 emulsion spray | 3.4 | 0.022 | 0.075 | 0.017 | 0.058 |
| 10% WPI spray/Myv 9-08 overspray | 1.6 | 0.011 | 0.018 | 0.012 | 0.019 |
| WPI powder/Myv 9-08 overspray | 1.8 | 0.007 | 0.013 | 0.010 | 0.018 |
| Myv 9-08 spray only | 1.1 | 0.014 | 0.015 | 0.013 | 0.014 |
| Uncoated ice control | — | 0.087 | — | 0.087 | — |
| Myv 9-08 coated ice control | 1.5 | 0.005 | 0.008 | 0.005 | 0.008 |

[a] Average thickness of the coating was calculated from weight of coating(s), total surface area of sample, density of the coating materials at 5–7°C, and assuming uniform coating.
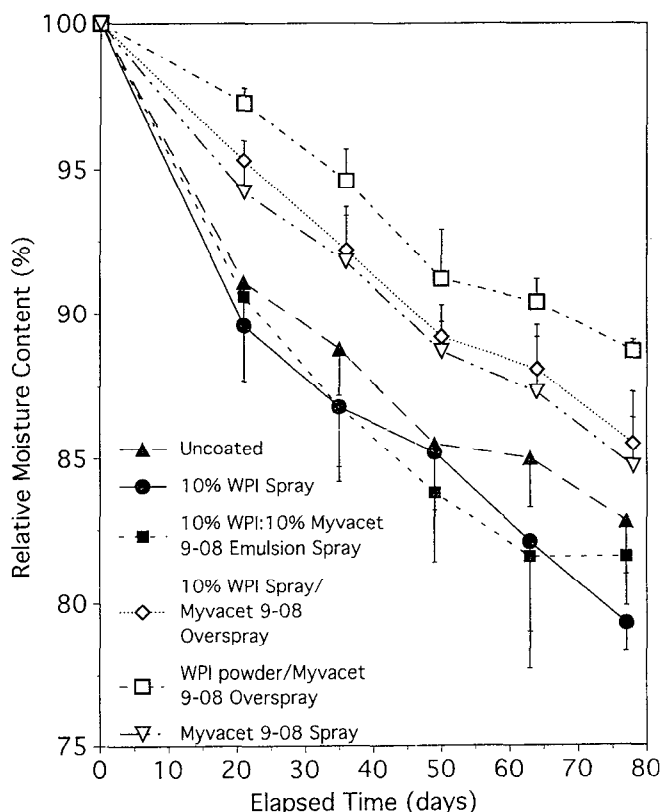


Fig. 2—Relative moisture content of frozen King salmon coated wtih WPI and acetylated monoglyceride edible coatings and stored at –23°C.

## RESULTS & DISCUSSION

### Model tests

Effects of WPI and acetylated monoglyceride coatings were compared on model gels over a 30-day test period (Fig. 1). The 10% WPI and 10% WPI:10% AMG emulsion coatings had moisture loss curves similar to uncoated gels. Coatings of AMGs and bilayer coatings of 10% WPI/AMG had much slower moisture loss compared to uncoated gels. The 10% WPI and AMG coatings were translucent, while emulsion coatings were milky in appearance.

### King salmon moisture loss test

Total thickness of coatings, calculated from weight of the coating(s), total surface areas, density of coating materials at 5–7°C, and assuming uniform coating were compared (Table 2). Effects of WPI and AMG coatings on relative moisture content of King salmon over 11 wks were compared (Fig. 2). Results were similar between the 10% WPI spray and 10% WPI spray/5% antioxidant overspray coatings and between Myvacet 9-08

and Myvacet 9-45 coatings with respect to moisture loss, thus data for antioxidant overspray and Myvacet 9-45 coatings were not included.

Moisture losses of fish samples coated with 10% WPI and 10% WPI:10% AMG emulsion coatings were comparable at all sampling times to uncoated samples. Average rates of moisture loss for these treatments were initially (0–3 wk) higher than those with an AMG spray or overspray, indicating that WPI and WPI emulsion coatings provided little moisture barrier at freezing temperatures (Table 2). Although the WPI emulsion coating was twice as thick as the WPI coating, it was initially (0–3 wk) only slightly more effective at reducing moisture loss. The average rates of moisture loss remained similar among uncoated, 10% WPI and WPI emulsion coated samples during the following 8 wk period, despite coating thickness differences (Table 2). Pham and Willix (1984) found that the desiccated surface layer, which remains behind as an ice front recedes during frozen storage of biological foodstuffs, produced a layer through which moisture must diffuse, causing resistance to mass transfer of moisture. A dessicated surface layer on the salmon formed during long storage times and was probably an additional barrier to moisture loss, resulting in lower rates of moisture loss for uncoated, WPI and emulsion coated fish during the last 8 wk of testing.

Samples coated with AMG spray or overspray had less (p<0.05) moisture loss than uncoated samples (Fig. 2). The WPI powder/Myvacet 9-08 overspray was a whitish coating, but had the lowest initial (0–3 wk) moisture loss rate per mm coating thickness (Table 2). This may have been due to a lower moisture coating on the surface of the fish, providing more initial resistance to moisture transfer. In addition, the dry protein coating probably provided a more uniform, less hydrophilic surface, allowing the AMG to bind more effectively to the surface. The 10% WPI spray/Myvacet 9-08 overspray coating, which was translucent, had higher initial moisture loss per mm coating thickness than the Myvacet 9-08 spray alone, which was almost transparent. During the last 8 wk period, as with the 10% WPI and WPI emulsion coatings, differences among treatments decreased with respect to rate of moisture loss (Table 2). The Myvacet 9-08 spray coating had the lowest moisture loss per mm coating thickness over this period.

Thus, edible film coatings consisting of AMGs alone or over protein coatings, were effective in reducing moisture loss. The effectiveness of low melting point AMG coating in slowing sublimation of ice suggested that it may be used to protect ice glazes on frozen fish.

### King salmon lipid oxidation test

Peroxide values (PV) of King salmon pieces coated with WPI and/or AMG during the 11 week study were compared (Table 3). PVs of the salmon pieces did not show significant differences until the fifth week of testing. At the fifth week, all coatings reduced the PV (p<0.10) compared to uncoated samples. The WPI powder/Myvacet 9-08 overspray coating had the lowest

Table 3—Peroxide values* (Meq/kg) of frozen King salmon pieces coated with WPI and acetylated monoglyceride coatings and stored at −23°C

| Treatment | Elapsed time (days) | | | | |
|---|---|---|---|---|---|
| | 21 | 35 | 49 | 63 | 77 |
| Uncoated | 1.67±0.79[a] | 3.69±0.91[a] | 2.70±0.50[abcd] | 2.34±0.95[ab] | 2.56±0.39[ab] |
| 10% WPI spray | 1.27±0.39[a] | 2.93±1.56[bc] | 2.73±0.20[abc] | 1.88±0.55[abc] | 2.36±0.92[abc] |
| 10% WPI spray/antioxidant overspray | 1.54±0.54[a] | 1.65±0.61[cde] | 2.65±0.96[abcde] | 1.74±0.58[abc] | 1.16±0.32[cd] |
| 10% WPI:10% Myv 9-08 emulsion | 1.48±0.69[a] | 1.50±0.34[cde] | 2.16±0.48[bcde] | 1.69±0.51[abc] | 1.84±0.60[bcd] |
| 10% WPI spray/Myv 9-08 overspray | 1.69±0.60[a] | 2.19±0.60[bc] | 2.45±0.51[abcde] | 1.57±0.40[bc] | 1.44±0.55[cd] |
| WPI Powder/Myv 9-08 overspray | 1.82±0.97[a] | 1.39±0.51[de] | 2.45±0.72[abcde] | 1.98±0.49[abc] | 1.81±0.16[bcd] |
| Myv 9-08 spray | 1.19±0.32[a] | 1.70±0.22[cde] | 2.12±0.94[cde] | 1.73±0.42[abc] | 1.69±1.04[bcd] |

* Initial peroxide value of salmon pieces was 1.34 ± 0.31 Meq/kg. Statistical analysis for the effect of treatment was performed separately for each elapsed time using Fisher's PLSD multiple comparison method.
[a-e] Treatments with the same letter within each elapsed time are not statistically different ($p \leq 0.10$).

PV. The order of effectiveness of remaining coatings was WPI: Myvacet 9-08 emulsion, WPI spray/antioxidant spray, Myvacet 9-08 spray only, WPI spray/Myvacet 9-08 overspray, with the WPI spray only coating having the greatest PV. At the sampling times of 7, 9, and 11 wk, difference in peroxide values between uncoated samples and the treatments was not as apparent, but almost all treatments were lower than the uncoated controls. In addition, the maximum peak in the PV was reduced and delayed 2 wk for coatings containing antioxidant or AMGs. This suggested that the coatings were effective in slowing diffusion of oxygen through the coating and reducing availability of oxygen to the fish surface. This may also be partially due to the decreased rate of moisture loss with AMG coatings. Banks and Hardy (1965) showed that greater drying loss resulted in higher peroxide values in the fat of 3.2 kg blocks of herrings stored for 6 mo. at −20°C.

Peroxide value is useful during the initial stages of lipid oxidation (Gray, 1978). In fish oils, the rate of hydroperoxide formation greatly exceeds its decomposition during early stages of lipid oxidation and correlates fairly well with oxidation (Smith et al., 1972). Decreases in peroxide values with time have been noted by other researchers. Awad et al. (1968) measured peroxide values in bovine muscle stored in 200g lots at −4°C for up to 8 wk. They found that the PV of beef decreased after 2 wk frozen storage and suggested that this was due to peroxide decomposition or interaction with protein. Noble (1976) measured PV of ground, deboned chicken meat stored in 0.9 kg lots at −23°C for up to 12 wks and found that the PV peaked at 6 wks, then decreased almost to original levels. Hirasa (1991) reported that the peroxide value correlated best with lipid oxidation in salmon when compared to thiobarbituric acid value and headspace gas chromatographic analysis for pentane.

The rate of lipid oxidation in fatty fish such as salmon is affected by several factors. The lipid content and composition of fish flesh can vary greatly depending on age, maturity, size of fish, and the position from where the sample was taken (i.e., head to tail, back or belly flap, dark or light meat) (Stansby and Olcott, 1963; Ackman, 1979; Flick et al., 1992). Fish such as salmon contain a wide variety of types of lipids, some highly unsaturated and highly reactive. The disposition of lipid within the tissue would also affect its rate of oxidation, with the dark muscled flesh tending to oxidize more rapidly than light flesh (Hardy, 1979). Nonlipid components, such as metals and tocopherols, can also affect lipid oxidation rates (Hardy, 1979). Oxidation increases when dehydration occurs (Banks, 1952). Hiremath (1973) showed that the main reason for oxidation of fats in frozen fish was dehydration of the tissue during storage and exposure to atmospheric oxygen. The effect of water activity on lipid oxidation is complex (Nelson and Labuza, 1992). Such complexity suggests that model systems with lipid components are needed to test the effects of edible coatings under frozen conditions.

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