Whey Protein and Acetylated Monoglyceride Edible Coatings: Effect on the Rancidity Process of Walnuts

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Whey protein isolate (WPI)-based coatings and distilled acetylated monoglyceride (AMG)-based coatings were evaluated for their ability to delay the oxidative rancidity of walnut pieces. Tocopherol (Toco) and ascorbyl palmitate (AP) were added to improve the performance of the coatings. Results indicate that, despite the low oxygen permeability and good continuity of the WPI coatings, there was no significant delay in the oxidative rancidity of walnuts. Shrinking of the WPI coating during drying, resulting in the pressing of some oil out of the walnut tissue, is likely the cause of the lack of effect of the WPI coatings. All AMG coatings resulted in increased shelf life of walnut pieces. After 70 days, the delay in the rancidity was as long as in the case of walnuts treated with butylated hydroxytoluene. Addition of Toco and AP in the AMG coatings had a positive effect in delaying the rancidity.

Keywords: *Whey protein; acetylated monoglycerides; coatings; walnuts; rancidity*

INTRODUCTION

Rancidity, the development of unacceptable off-flavors resulting from oxidation of lipids, is a major problem in walnuts during storage (Rockland et al., 1961). Persian walnut kernels are sensitive to oxidation because of their high oil content (≈65% w/w dry basis) and high polyunsaturated fatty acid content (>90% of total fatty acids in some varieties) (Greeve et al., 1992). Furthermore, walnut kernel pieces are more susceptible to lipid oxidation because of the increased surface area and the injuries caused by the breaking process.

Lipid oxidation has been studied extensively in the past (Vercellotti et al., 1992; Labuza, 1971). Environmental factors such as temperature (Wright, 1941), oxygen concentration (Maté et al., 1996a; U.S. Department of Agriculture, 1959; Wright, 1941), relative humidity (Rockland et al., 1961; Swarthout et al., 1958), and light (U.S. Department of Agriculture, 1959), as well as processing factors such as shelling (Wright, 1941) and the addition of antioxidants (Rockland et al., 1961; Swarthout et al., 1958) have been shown to affect lipid oxidation of walnuts.

Nitrogen flushing in low oxygen permeability packaging and the addition of synthetic antioxidants are used successfully in the industry as means to increase the shelf life of walnuts (Walnut Marketing Board, 1993). The use of edible coatings provides an alternative approach. Reduced O_2 content within each nut could be achieved by surrounding each nut with a continuous edible coating with a low O_2 permeability. In addition, edible coatings could be used as carriers of natural antioxidants. This would allow controlled release of antioxidants on the surface, where oxidation is more likely.

Whey protein isolate (WPI)/glycerol (Gly) edible films have low O_2 permeability (McHugh and Krochta, 1994). When used on dry-roasted peanuts as coatings, they delayed oxidative rancidity (Maté and Krochta, 1996; Maté et al., 1996b). In addition, it was shown that the mechanism of protection by the coatings was their effect as oxygen barriers. As a consequence of the previous work, it was hypothesized that WPI/Gly coatings could have the same outcome on walnut pieces. The effect of WPI coatings on walnuts could be enhanced by the addition of antioxidants to the coating solution. This was shown by Swenson et al. (1953), who increased the ability of pectin-based coatings to delay the rancidity of almonds by adding synthetic antioxidants to the coating formulation.

Butylated hydroxytoluene (BHT) is currently being used successfully as an antioxidant by the walnut processing industry (Walnut Marketing Board, 1993). However, since this is a synthetic antioxidant, consumers have expressed a desire to eliminate it (Finley and Otterburn, 1993). Tocopherols, very efficient natural antioxidants widely used in the food industry (Schuler, 1990), could be used as an alternative. There are four chemically different tocopherols: α, β, γ, and δ. The order of their antioxidant activity is $\delta > \gamma > \beta > \alpha$. In addition, the use of a synergist could enhance the action of the tocopherols and reduce costs. Ascorbyl palmitate (AP) has shown a synergistic effect with tocopherols (Dziezak, 1986). Furthermore, the combination of AP and tocopherols in ethanol was shown to delay the rancidity process in nuts (Schuler, 1990).

Acetylated monoglycerides (AMG) have been studied in the past as water vapor barrier coatings on foods to retard dehydration (Woodmansee and Abbot, 1958; Clark and Shirk, 1965; Luce, 1967; Alikonis, 1979; Stuchell and Krochta, 1995). AMG films were shown to have high oxygen permeability, similar to that of low-density polyethylene (Luce, 1967). Hoover and Nathan (1981) showed that AMG coatings did not provide protection against lipid oxidation of granulated roasted peanuts. However, there is a possibility of using AMG coatings as an antioxidant carrier. This was pointed out soon after acetoglycerides were developed (Feuge, 1955). In this regard, the advantage of AMG coatings over WPI coatings is the higher hydrophobicity of AMG. Some of the most effective natural antioxidants such

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as tocopherols are highly hydrophobic. Since an AMG coating is quite hydrophobic compared to a WPI coating, tocopherols would migrate more freely to the nut surface in an AMG coating compared to a WPI coating. Thus, AMG could be more effective as a carrier of antioxidants.

The goal of this research was to test WPI- and AMG-based coatings as means to delay oxidative rancidity in walnut pieces. To achieve this goal, walnut pieces were coated with different coating formulations and their effect was tested using an accelerated rancidity method (Maté et al., 1996a).

MATERIALS AND METHODS

Materials. Shelled Persian walnut pieces (light medium pieces) and BHT in corn oil solution (20% w/w) were supplied by Diamond Walnut Growers, Inc. (Stockton, CA). Nuts were sent to UC Davis overnight and kept at 2 °C until used. Fresh corn oil was obtained from a local supermarket. WPI (>95% protein) was obtained from Davisco Foods International (Le Sueur, MN). AMG Myvacet 5-07 was supplied by Eastman Chemical Products, Inc. (Kingsport, TN). Gly and sodium bromide were obtained from Fisher Scientific, Inc. (Fair Lawn, NJ). Tocopherol (Toco) concentrate COVI-OX T-70 (typical composition: α-Toco, 8%; β-Toco, 1%; γ-Toco, 42%; and δ-Toco, 18%) was supplied by Helken Corp. (LaGrange, IL). AP was obtained from Sigma Chemical Co. (St. Louis, MO).

WPI Solution. The method developed by McHugh et al. (1994) to prepare WPI edible film solutions was used with the modifications described by Maté and Krochta (1996) to increase solution viscosity. Aqueous solutions of 11% WPI (w/w) were prepared and degassed under vacuum. The solution was maintained at 90 °C for 30 min in a water bath, followed by cooling to room temperature. Gly was then added to the solution as a coating plasticizer until there was a 60/40 (w/w) ratio of WPI and Gly (solution WPI60/Gly40). Solutions were again degassed and stored at 2 °C for 7 days to allow viscosity to increase.

WPI/Toco Emulsions. Tocopherols were incorporated into the coating solution by taking advantage of the emulsifying ability of WPI. A concentrated Toco emulsion was created and then diluted to the desired Toco concentration using high-viscosity WPI solution. First, a WPI60/Gly40 was prepared as described above, but without the storage period. COVI-OX T-70 was added to the solution until the concentration was 10% Toco (w/w). To make an emulsion, the mixture was first prehomogenized using an Ultra-Turrax T-25 homogenizer Model 125 (IKA-Works, Inc., Cincinnati, OH) for 2 min at 10 000 rpm, followed by 6 min at 23 000 rpm. The mixture was then homogenized by using two passes at 10 000 psi through a high-pressure laboratory homogenizer (Rannie 8.30H, APV Rannie Inc., St. Paul, MN). This concentrated Toco emulsion was diluted to either 1.1% or 0.11% Toco (w/w) using high-viscosity WPI60/Gly40 solution (prepared as described above). The ratios between concentration of antioxidant and whey protein selected were similar to the ratios between concentration of antioxidant and pectin used by Swenson et al. (1953) to successfully delay oxidative rancidity in almonds with pectin coatings.

WPI Coating Procedure. The method used by Maté and Krochta (1996) to coat dry-roasted peanuts with a WPI solution with increased viscosity was used. Walnut pieces were first cleaned with a brush to remove small pieces and dust and then placed on pins that were attached to a board. Then, the nut pieces were dipped into the selected WPI coating solution for about 1 s. The solution was maintained at 5 °C by an ice bath. Immediately after, the pin boards with nuts were placed in a dryer at room temperature for 10 min. The boards were automatically rotated to get an even distribution of the coating around the nuts. Nuts were coated with three layers of WPI solution (either WPI60/Gly40, WPI60/Gly40 + 1.1% Toco, or WPI60/Gly40 + 0.11% Toco). Three layers were necessary to obtain a continuous coating around the nut. After the last coating was applied, nuts were placed in the dryer for 30 min to get more complete drying. After this time, walnut pieces were taken from the boards and the small hole made by the pin in each walnut piece was covered with a drop of solution followed by drying. Controls (uncoated walnut pieces) went through the same procedure except for the dipping step.

AMG Formulations and Coating Procedure. Two levels (high and low) of tocopherols in the AMG coatings were studied. High level and low level corresponded to 14% and 1.4% (w/w) of Toco in the AMG solution, respectively (high Toco−no AP and low Toco−no AP treatments). To study the effect of AP as a synergist of Toco, two other treatments were prepared with the same amount of Toco and with added AP at a 1:1 mole ratio with respect to the amount of Toco. Since the molecular weights of γ-Toco and AP are very similar (416.7 and 414.5, respectively), the treatment ended up with 14% Toco + 14% AP (high Toco−yes AP) and 1.4% Toco + 1.4% AP (low Toco−yes AP). To make a complete study, two controls were prepared: AMG without any other additive (no Toco−no AP) and AMG with 14% AP (no Toco−yes AP).

A mixture of the required amounts of AMG, Toco concentrate, and AP was added to a beaker. This beaker was then placed in a water bath at 75 °C to melt the AMG. The mixture was stirred with a magnetic stirring bar under a nitrogen flow to decrease oxidation of the mixture. Brush-cleaned walnut pieces were placed on pins and dipped in liquid AMG mixture for about 1 s. After cooling, walnut pieces had a thin layer of solid AMG coating. This procedure is similar to the one described by the company that produces the AMG (Eastman Chemical Products, 1987). Controls (uncoated walnut pieces) also went through the same procedure, except for the dipping step.

BHT Treatment. Walnut pieces were treated with a BHT solution to simulate a common treatment used in the industry. A 2.55% (w/w) solution of BHT in corn oil was prepared. This solution was sprayed on brush-cleaned walnut pieces using an aerosol sprayer (spra-tool) from Crown Industrial Products Co. (Hebron, IL). The walnuts were shaken while spraying to increase the homogeneity of the treatment. The increase of weight of walnuts due to the treatment was monitored. The spraying was stopped when there was an increase in weight of 0.55 ± 0.05%. This increase corresponded to a treatment of about 200 ppm of added BHT with respect to walnut lipid content.

Equilibration Procedure. After the coating solutions were applied and once the coatings were dry or solidified, coated and uncoated walnut pieces were placed in individual glass containers to be equilibrated at 53% relative humidity, a common relative humidity used to store walnuts (Walnut Marketing Board, 1993). Constant relative humidities were maintained by saturated salt solutions in the bottom of glass containers (0.75 L) with airtight lids. Nuts were suspended over the salt solution on a nylon screen. Sodium bromide (NaBr) provided a relative humidity of 53% at 37 °C (Kitic et al., 1986). Preparation of the solutions was performed according to standards (ASTM, 1985).

The glass containers with the walnut pieces were placed into a controlled-temperature room at 37 °C. All glass containers were flushed with nitrogen weekly to prevent lipid oxidation during the equilibration period. Light was excluded by covering all containers with aluminum foil. Earlier experiments showed that after 3 weeks, walnuts could be considered at equilibrium with the selected relative humidity (Maté et al., 1996a).

Weight Increase. The amount of WPI coating added to the walnut pieces was calculated by measuring the difference in weight between 10 walnut pieces before coating and after the equilibrium period, taking into account the change in weight of uncoated walnut pieces because of the relative humidity. The amount of AMG coating added was determined by measuring the difference in weight between 10 walnut pieces before and after coating.

Storage Test. After the equilibration period, walnut pieces were transferred from individual equilibration containers to individual oxidation containers (0.75 L) with the same saturated salt solution. For all AMG coating treatments and controls, duplicate oxidation containers were used. One oxidation container per each WPI coating treatment was used. The

Table 1. Amount of Coating Added to the Nut Meat in the Different Treatments

WPI treatment	coating wt[a] (%)	AMG treatment	coating wt[a] (%)
WPI60/Gly40	22.30	AMG, no Toco, no AP	4.38
WPI60/Gly40 + 1.1% Toco	26.09	AMG, no Toco, 14% AP	4.75
WPI60/Gly40 + 0.11% Toco	20.91	AMG, 14% Toco, no AP	3.58
		AMG, 14% Toco, 14% AP	3.18
		AMG, 1.4% Toco, no AP	2.80
		AMG, 1.4% Toco, 1.4% AP	4.91

[a] Coating weight based on amount of coating in final weight of coated nut.

oxidation containers had air in the headspace to allow lipid oxidation and were kept at 37 °C. A sample containing 10 walnut pieces (7 g) was collected from each container to determine the initial oxidation level. Samples were then collected every 2 weeks for 10 weeks to follow the progression of the rancidity process. Static head space analysis was performed in duplicate with all samples.

Static Headspace Analysis. The method described by Frankel and Huang (1994) to analyze static headspace for oil and slightly modified for nut samples (Maté et al., 1996a) was used for determining hexanal content.

RESULTS AND DISCUSSION

Evaluation of the Effect of WPI-Based Coating. WPI coatings provided a glossy appearance to the walnut pieces. Irregularities on the nut surface were smoothed by the WPI coating, creating a distinctive candylike appearance. In addition, there was a darkening effect during the storage test. This darkening effect was also observed in peanuts treated with WPI-based coatings (Maté et al., 1996b), but it was less intense than in the case of coated walnuts.

The amount of WPI coating added to the walnut pieces varied between 20 and 25% (Table 1). In addition, the coatings were not homogeneously distributed on the nut surface because of the surface irregularities and the high viscosity of the WPI solution.

It was observed that there were small amounts of a viscous liquid between the coating and the nut surface. That liquid was thought to be either walnut oil from the nut tissue or Gly from the coating. Since it was not soluble in water, it was assumed to be walnut oil. It was observed that the WPI coating shrank during drying because of the continuous loss of water. This phenomenon continued during the equilibration period. Probably due to the shrinkage, the WPI coating exerted some pressure on the nut meat and squeezed oil out of the tissue. This process was also observed when WPI coatings were applied to dry-roasted peanuts (Maté et al., 1996b). The amount of oil was not quantified, but it was apparently greater than in the case of the peanuts, probably because dry-roasted peanuts have stronger structure and less oil content.

The effect of the WPI coatings on oxidative rancidity is shown in Figure 1. The induction period of a rancidity process is defined as the period of time during which there is not detectable off-flavor development (Diplock, 1993). In the present study, the induction period was considered as the time required for an exponential increase in hexanal content in the samples. Walnut pieces coated with WPI60/Gly40 + 1.1% Toco or WPI60/Gly40 + 0.11% Toco, as well as untreated walnuts, had an induction period of 21 days. On the other hand, walnuts treated with WPI60/Gly40 coatings (no antioxidants) had a shorter induction period (Figure 1).

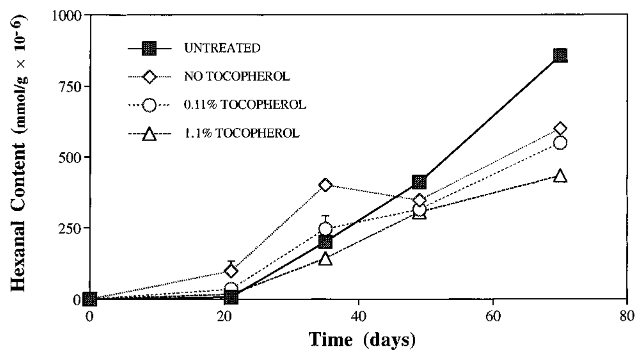

Figure 1. Effect of WPI-based coatings on the hexanal content (static headspace analysis) of walnut pieces. Bars represent standard deviations.

Swenson et al. (1953) found that pectin-based coatings had a negative effect on the oxidative rancidity of almonds. They claimed that the catalytic action of coating impurities was the cause of such results. In our case, the disturbances caused by the shrinkage of the coating on the walnut surface were likely responsible for the negative effect of the WPI coatings. These disturbances forced some oil out of the tissue and probably activated the enzymatic mechanism responsible for lipid oxidation in walnuts (Greeve and Labavitch, 1985). Injuries in plant tissue during processing can activate other enzymatic systems such those responsible for enzymatic browning (Rouet-Mayer et al., 1993; Mohsenin, 1986). After the coating shrinkage, the walnut pieces were more susceptible to lipid oxidation and the decreased oxygen concentration due to the WPI coating was not enough protection to delay the rancidity. The antioxidants present in the coating contributed to increase of the stability of coated walnuts, resulting in longer induction period. However, this induction period was not longer than the induction period of untreated walnuts (Figure 1).

Evaluation of the Effect of AMG-Based Coating. AMG coatings were chosen as an alternative carrier of antioxidants for two reasons: First, these coatings do not shrink on the nut surface, and therefore no oil is pressed out of the tissue. Second, antioxidants can migrate more freely in AMG medium than in the WPI emulsion because of the higher hydrophobicity of the AMG coatings; thus, antioxidants could be more readily available on the nut surface.

Walnut pieces coated with AMG coatings looked very similar to untreated walnuts. The treatments with 14% AP (no Toco−yes AP and high Toco−yes AP) looked slightly more orange than untreated walnuts.

Walnuts pieces were coated with just one layer of AMG solution, since this was enough to create a continuous thin layer on the surface of the nutmeat. As opposed to WPI coatings, AMG coatings were very homogeneous despite the irregularities of the walnut surface. The amount of AMG coating added to the walnut varied from 2.8 to 5% (Table 1). Differences in viscosity and surface tension of the AMG solutions at 75 °C are likely to be the cause of the differences in amount of coating on the walnut surface.

The effect of AMG-based coating on the oxidative rancidity of walnuts pieces is shown in Figure 2. All of the treatments, regardless of amount of tocopherols or AP, had a positive effect in delaying the rancidity. Untreated walnuts had an induction period of about 21 days. Walnut treated with no Toco−no AP coating had an induction period of about 56 days. The rest of the

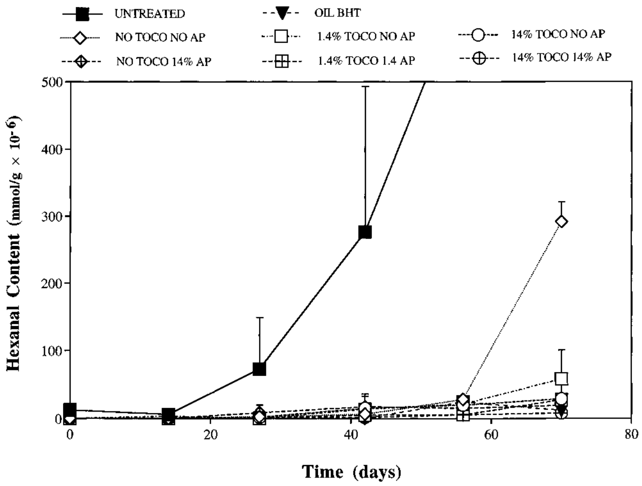

Figure 2. Effect of AMG-based coatings on the hexanal content (static headspace analysis) of walnut pieces. Bars represent standard deviations.

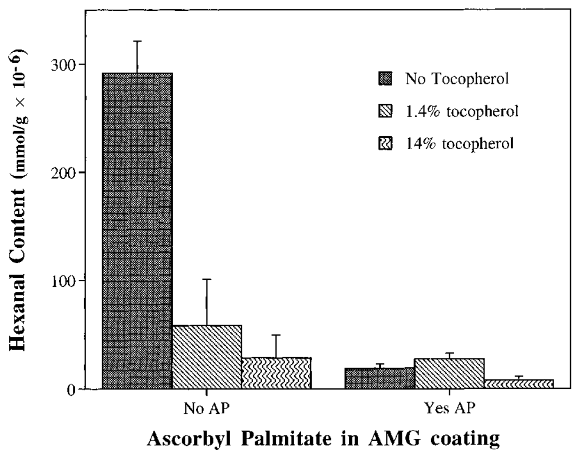

Figure 3. Effect of AP and Toco on the hexanal content (static headspace analysis) of walnut pieces coated with AMG-based solutions on the 70th day of the experiment. Bars represent standard deviations.

AMG treatments, as well as BHT treatment, had an induction period >70 days, the end point of the experiment.

AMG obtained commercially as Myvacet 5-07 from Eastman Chemical Products, Inc. (Kingsport, TN), had some citric acid in its formulation (<0.1%). Citric acid is a chelating agent (Dziesak, 1986) added to reduce the lipid oxidation of AMG. This small amount of citric acid on the walnut surface could have contributed to reduction of the oxidative rancidity of the walnuts and could explain the positive effect of AMG coatings without antioxidant agents. However, Hoover and Nathan (1981) showed that the same AMG coating did not provide any positive effect on the rancidity of granulated roasted peanuts. Granulated roasted peanuts have larger surface area due to smaller size and porosity. Probably, AMG coatings were not applied thoroughly on all granulated nut surfaces. In our case, the walnut piece surfaces were completely covered by a thin layer of AMG coating.

Analysis of variance (ANOVA) of the hexanal content for the different AMG treatments at the 70th day was conducted to evaluate the effect of the presence of tocopherols or AP on the performance of the coatings (Figure 3). Results from ANOVA indicated that there was a significant positive effect of the addition of tocopherols into the formulation ($p < 0.05$). Furthermore, the hexanal content of high-Toco treatments was significantly lower that that of low-Toco treatments ($p < 0.05$). It is expected that in longer experiments, the differences between these two treatments would be larger. The presence of AP also contributed significantly to decreased hexanal content at the 70th day ($p < 0.05$) and so to the delay of the rancidity (Figure 3). It is important to point out that the addition of AP without tocopherols caused a significant rancidity delay. The combined effect of AP together with small amounts of citric acid and the natural tocopherols present in the walnut tissue probably was the reason for this result. The effect of the presence of either tocopherols or AP was so large in this experiment that it was not possible to test the possible synergistic effect. Longer experiments with smaller amounts of antioxidants are necessary to optimize the AMG coating formulation.

Conclusions. WPI coatings, applied as viscous solution, were found not to have the expected positive effect on the oxidative rancidity of walnut pieces. Shrinking of the WPI coating during drying caused disturbances in the walnut tissue that likely made the walnut pieces more vulnerable to oxidation. The addition of tocopherols as antioxidant into the WPI coating formulas resulted in a longer shelf life, but it was not significantly different from that of untreated walnut pieces. To use the low O_2 permeability properties of WPI films in walnuts, it would be necessary to explore other coating approaches that do not produce shrinking effects.

On the other hand, AMG coatings on walnut pieces resulted in increased shelf life. After 70 days, results indicated that the presence of both tocopherols and AP had a significantly positive effect on the performance of the AMG coating. The presence of citric acid in the formulation of the AMG coating is likely to contribute to the positive effect of AMG coatings without antioxidants. With AMG coatings, walnut rancidity was delayed by supplying antioxidant agents to the surface. These experiments showed that by controlling lipid oxidation on the surface, where it is more active, rancidity of the whole nut could be successfully controlled.

ABBREVIATIONS USED

WPI, whey protein isolate; AMG, distilled acetylated monoglycerides; BHT, butylated hydroxytoluene; AP, ascorbyl palmitate; Gly, glycerol; Toco, tocopherol.

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