

# The Gloss of Edible Coatings as Affected by Surfactants, Lipids, Relative Humidity, and Time

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**ABSTRACT:** Water-based whey protein isolate (WPI) coatings had the same gloss as shellac and zein coatings cast from ethanol solutions and water-based tapioca dextrin and hydroxypropyl methylcellulose (HPMC) coatings. For lipid dispersion coatings, the particle size of the dispersed phased influenced gloss values. WPI coatings had stable gloss values at 52%, 75%, and 95% relative humidity (RH). At 95% RH, shellac and zein coatings “blushed,” and both dextrin and HPMC coatings became tacky. WPI and shellac films maintained a high gloss after 7.6 mo of storage at 23 °C and 75% RH and had higher gloss than whey protein concentrate (WPC) and HPMC coatings. The type and level of surfactant added to WPI coatings greatly influenced gloss.

**Key Words:** edible coatings, gloss, surface properties, emulsions, surfactant

## Introduction

### Edible coatings and gloss

Because of increased regulation of the emission of volatile organic compounds (VOCs) into the atmosphere by confectionery manufacturers, water-based alternatives to the commonly used VOC-containing coatings are necessary (Anon 1998). The level of VOCs permitted to be released into the atmosphere is gradually being reduced (with an ultimate goal of zero discharge) and the U.S. government is issuing large fines to firms who are in noncompliance with VOC emission standards (Glesecke 1998). Panned confectionery products, such as chocolate- and sugar-panned products, are typically finish coated to add an aesthetically-pleasing gloss (Jordan 1992). The most common polishing agents used in chocolate panned confectionery items are ethanol-based shellac and corn zein coatings (Boutin 1997; Groves 1992; Freeman year unknown). Ethanol and additives, such as ethyl acetate, that are used for shellac coating formulations for confections are VOCs.

Water-based whey protein (WP) coatings have the potential to replace ethanol-based shellac and corn zein coatings for panned confectionery products. These water-based WP coatings form water-insoluble, flexible, transparent, coatings that are bland (McHugh and Krochta 1994a; Krochta and McHugh 1996; Miller and others 1998). WP coatings also have excellent oxygen-, aroma-, and oil-barrier properties (Maté and Krochta 1996; McHugh and Krochta 1994b; Krochta 1997; Maté and others 1996); however, their gloss properties are not known. High-gloss WP coatings could also replace the dextrin/gum Arabic precoatings, which are often used as an ethanol barrier before the shellac coatings are applied, or as tack reducing and gloss modifying additives in sugar coatings. Thus, WP coatings could drastically lower VOC emissions by confectionery manufacturers and reduce the number of coatings steps necessary for certain products. The objectives of this study were to determine the gloss properties of water-based WP coatings compared to commercial ethanol-based shellac and corn zein coatings.

### Milk proteins: allergen and kosher concerns

Whey protein is a component of milk. Milk is one of the most common allergenic foods in North America, especially in children (Taylor 1996). Confections that contain milk chocolate or yogurt are already avoided by people sensitive to milk. Thus, WP coat-

ings are appropriate on these types of products.

Another issue related to the use of milk protein is the kosher certification of foods. The U.S. kosher food market produces about \$35 billion in sales annually and spans across more than just the Jewish consumer market (Regenstein and Regenstein 1996). Jewish religious law forbids the mixing of meat and milk. Within the realm of milk chocolate-containing items, where WP coatings are an ideal fit, the kosher impact is negligible. For new products that contain no milk products, marketing studies could determine the acceptability of a milk-protein coating.

## Results and Discussion

### Gloss and haze of lipid-free coatings

After 16 h of drying, coatings were not tacky. Qualitatively, all coatings except hydroxypropyl methylcellulose (HPMC) had a high gloss and gave a clear reflection. Shellac, zein, whey protein isolate (WPI), and dextrin were high gloss coatings since  $G^{60}$  values were  $> 70$  (Table 1). The gloss ( $G^{60}$  and  $G^{20}$ ) values of HPMC coatings were less those of the other 4 materials (Table 1).

Relative humidity (RH) had a small effect on coating gloss but a large effect on coating quality. At 23% RH, HPMC, zein, and dextrin coatings cracked and de-adhered from the plastic sheets. The plasticizer level necessary to achieve intact coatings from these materials at 23% RH caused the coatings to be tacky and unacceptable. Dextrin coatings also cracked after 2 to 3 d at 52% RH. The  $G^{60}$  gloss values of zein, WPI, shellac, and HPMC coatings did not change after 2 wk of storage at 52% RH (Fig. 1).

The higher RH values tested made it difficult to accurately measure gloss. After 40 h at 75% RH, dextrin and HPMC became tacky with reduced gloss. The gloss meter would stick to the surfaces and cause pull marks when removed. The altering of the surface composition due to moisture absorption may have also contributed to the reduced gloss of HPMC coatings (since these HPMC coatings were not as tacky as dextrin coatings at 75% RH). At 95% RH, the quality of all the coatings was relatively poor. Dextrin films became liquefied, and HPMC films were very tacky. After 6 d at 95% RH, WPI films became moldy, and gloss could not be measured. Shellac and zein coatings “blushed” (turned white) after a few hours at 95% RH, as seen by a large increase in whiteness index (WI) values (Table 2). WPI coatings did not blush nor were they tacky.

**Table 1—Gloss of lipid-free edible coatings after drying 16 h under ambient conditions**

Coating	Number of samples	Mean G <sup>60</sup>	Mean G <sup>20</sup>
Shellac	9	93 <sup>a</sup> (0.35)	76 <sup>A</sup> (3.6)
Zein	3	92 <sup>a</sup> (0.44)	55 <sup>A</sup> (3.5)
WPI	13	91 <sup>a</sup> (1.4)	72 <sup>A</sup> (17)
Dextrin	4	84 <sup>a</sup> (10)	68 <sup>A</sup> (3.8)
HPMC	4	64 <sup>b</sup> (2.7)	30 <sup>B</sup> (7.2)

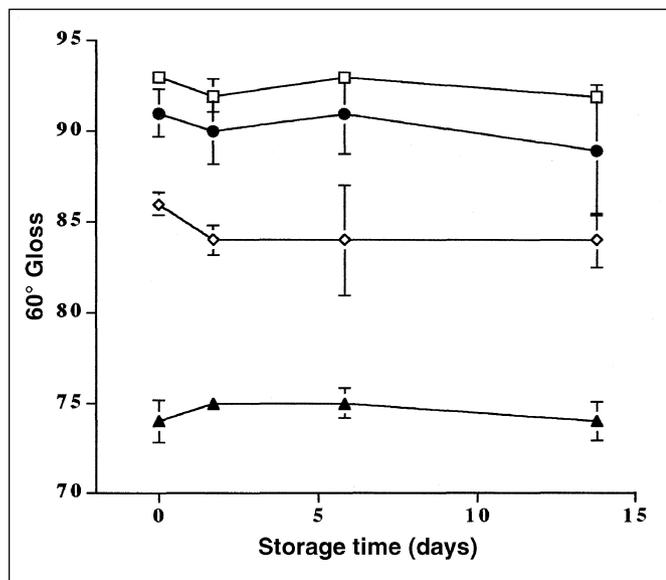
Means with the same superscript within the same column are not different at  $p = 0.001$  and  $p = 0.0381$  for G<sup>60</sup> and G<sup>20</sup> values, respectively. Values in parentheses are the standard errors about the means.

### Effect of surfactants on WPI coating gloss

Reliable gloss values of lecithin-containing coatings were not obtained because the coatings peeled off the plates during drying and conditioning. The resultant coating surfaces were not flat. Span 20 had little effect on G<sup>60</sup> values until an amount of more than 2.7% (dry coating basis) was added to the coating (Fig. 2). Tween 85 reduced G<sup>60</sup> values when more than 1% was added to the coatings. It is not clear why Tween 85 had such a dramatic influence on WPI coating gloss. This result may be related to the size of the surfactant molecules. The average formula weights of Tween 85 and Span 20 are 1839 and 346 g·mol<sup>-1</sup>, respectively (Aldrich 1996). Molecules of Tween 85 may be too large to mix with the polymer-glycerol system as the coating dried. Thus, Tween 85 molecules aggregate and may migrate to the coating-air interface. At higher surfactant concentrations, opaque clusters or aggregates were noticed on coatings containing Span 20 or Tween 85. Fina and others (1992) found that the nonionic surfactant Triton X-100, a poly-disperse poly(ethylene oxide)-based surfactant, migrated to the surface during the drying of gelatin films. Migration continued for months after initial drying.

### Lipid-dispersion coatings

The G<sup>60</sup> values for WPI- and shellac-dispersion coatings were dramatically influenced by lipid content and particle size. WPI-AMF coatings with a low dispersed phase particle Sauter mean diameter (SMD; 0.39 μm) maintained G<sup>60</sup> values that were greater than 70 when the film contained up to 40% lipid (Table 3). In



**Fig. 1—60° gloss values of edible coatings when stored at 23°C and 52% RH. Key: □ = shellac, ◇ = WPI, ● = zein, and ▲ = HPMC**

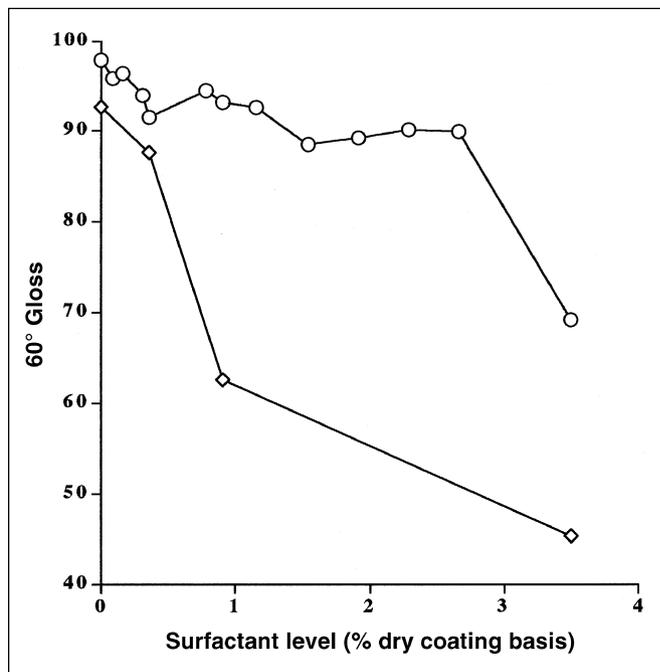
**Table 2—Whiteness index (WI) of edible coatings initially after drying (day 0) and after storage at 75% and 95% RH at 23 °C**

Coating	75% RH		95% RH	
	Day 0	Day 13	Day #	WI
WPI	5.3	6.5	5	7.1
Shellac	5.9	7.2	< 0.25	29.6
Dextrin	7.5	6.3	0	n/a
Zein	8.9	12	< 0.25	37.5
HPMC	19.8	24.2	0	n/a

comparison, WPI-carnauba-wax (CW) and shellac-CW coatings with large dispersed phase particle SMD values of 1.4 to 1.6 μm, dramatically lost gloss as lipid content of the coating increased (Table 3).

The shape of the dispersed phase particle size distributions also influenced dispersion-coating gloss. Shellac-CW coatings had a more uniform particle size distribution than WPI-CW coatings, as shown by lower span, D[v, 0.5] and D[v, 0.9] values (Table 4). Even though the WPI-CW and shellac-CW coatings had similar SMD values, the more uniform distribution resulted in higher gloss values of shellac-CW coatings. WPI-AMF coatings had relatively tall and narrow particle size distributions. The small SMD of 0.39 μm created a stable dispersion. This prevented most of the lipid particles from reaching the air-coating interface, giving these coatings a high gloss. At low dispersed particle SMD values (approximately < 1.0 μm), WPI-lipid emulsions are considered stable to phase separation (Shellhammer and Krochta 1997). Time had virtually no effect on the gloss of WPI- and shellac-dispersion coatings stored for almost 5 mo at 75% RH and 23 °C (Table 3).

After storage of coatings at 23 °C and 75% RH for 7.6 mo, lipid-free WPI and shellac coatings had greater gloss and lower haze values than whey protein concentrate (WPC) or HPMC coatings



**Fig. 2—The effect of surfactants on 60° gloss values of WPI coatings. Key: ◇ = Tween 85, □ = Span 20. Drying was accomplished for 24 hours at ambient conditions.**

**Table 3—The effect of lipid content and storage time (23 °C, 75% RH) on the gloss of lipid-containing edible biopolymer coatings**

Coating	storage time (months)	60 ° Gloss at % lipid in coating (dry basis)			
		0	10	20	40
WPI + CW	0	86 (2.3)	12 (0.9)	6 (0.1)	3 (0.1)
	0.57	85 (1.1)	16 (1.0)	7 (0.3)	3 (0.1)
	1.3	86 (3.0)	15 (0.4)	6 (0.4)	3 (0.1)
	2.5	88 (2.9)	14 (0.7)	6 (0.7)	3 (0.1)
	4.9	85 (2.8)	14 (0.8)	6 (0.8)	3 (0.1)
WPI + AMF	0	97 (3.1)	78 (2.4)	77 (1.8)	76 (0.7)
	0.17	92 (0.8)	73 (1.8)	63 (2.5)	61 (7.0)
	0.67	91 (0.7)	72 (1.3)	65 (3.4)	62 (7.0)
	1.9	91 (1.0)	73 (1.8)	67 (5.2)	62 (4.0)
	4.3	91 (5.2)	75 (1.5)	69 (3.5)	64 (4.0)
Shellac + CW	0	93 (0.3)	53 (3.2)	44 (3.4)	19 (1.9)
	0.40	92 (0.6)	46 (3.3)	38 (2.8)	18 (1.7)
	0.97	92 (0.7)	49 (5.4)	38 (3.2)	18 (3.7)
	1.7	98 (0.8)	47 (5.5)	36 (2.2)	18 (3.8)
	2.9	94 (1.3)	50 (5.5)	37 (2.6)	18 (4.0)
5.5	96 (0.9)	53 (5.8)	40 (2.0)	17 (5.0)	

The SMDs of the dispersed phases for WPI-AMF, WPI-CW and Shellac-CW coatings were 0.39  $\mu$ m, 1.4  $\mu$ m and 1.6  $\mu$ m, respectively. (Standard deviations are in parentheses.)

(Table 5). HPMC and WPC coatings both had low gloss ( $G^{60} < 70$ ) after 7.6 mo; while, shellac and WPI coatings had exceptionally high gloss ( $G^{60} \geq 90$ ) after the same period. The decreased gloss and increased haze properties of WPC coating compared to shellac and WPI coatings were probably due to the 3.9% lipid in the WPC coatings. The dispersed lipid particles may have created heterogeneous and rough surfaces that influence coating gloss.

The  $G^{60}$  values of WPC coatings were generally less than those of WPI-AMF coatings and greater than WPI-CW and shellac-CW coatings at the 10% lipid level (Tables 3 and 5). These results are caused by the particle size distribution of the WPC coatings. The SMD,  $D[v,0.5]$ , and span parameters of the WPC coatings were generally between those of the WPI-AMF and CW-containing coatings (Table 4). Since lipid levels from 10% to 40% did not lower gloss values, it appears that the lipid particle size distribution

**Table 4—Parameters of the lipid particle size distributions of WPI-carnauba wax, Shellac-carnauba wax, WPI-AMF and WPC dispersion coatings**

Coating	D[3,2]	D[v,0.9]	D[v,0.5]	span
WPI-AMF	0.39	2.4	0.44	5.1
WPI-CW	1.4	23	2.5	8.9
Shellac-CW	1.6	5.2	2.0	2.1
WPC	0.87	6.9	1.2	5.7

Particle sizes are in micrometers.

**Table 5—Mean  $G^{60}$ ,  $G^{20}$ , and Haze Index (H) values of edible coatings after storage for 7.6 months at 23 °C and 75% RH**

Coating	$G^{20}$	$G^{60}$	H
WPI	80 <sup>a</sup>	90 <sup>1</sup>	10*
Shellac	72 <sup>a</sup>	91 <sup>1</sup>	19*
WPC	38 <sup>b</sup>	66 <sup>2</sup>	28‡
HPMC	34 <sup>b</sup>	63 <sup>2</sup>	29‡

$G^{20}$ : means with the same letter are not different ( $p = 0.0047$ ).

$G^{60}$ : means with the same number are not different ( $p = 0.044$ ).

H: means with the same symbol are different ( $p = 0.0011$ ).

had the greatest influence on lipid-dispersion coating gloss.

## Conclusions

WPI AND SHELLAC COATINGS PRODUCED A HIGH GLOSS that is desirable for many food products. Depending on the surfactant type, gloss values of WPI coatings were unchanged or dramatically lowered. The gloss of lipid dispersion coatings was high when the particle size of the lipid was low. The distribution of lipid particles also influences gloss of lipid-containing coatings. Storage time did not affect lipid-free or dispersion-coating gloss. WPI and shellac coatings had superior gloss retention properties at 23 °C and 75% relative humidity for over 7 mo of storage. Overall, the gloss properties of WPI are promising for use as a shellac replacement for confectionery coatings.

## Materials and Methods

COATINGS WERE MADE FROM LIQUID SOLUTIONS OR DISPERSIONS. All ingredients were food grade, meeting Food Chemical Codex requirements. Coating formulations were typical of those reported for use as coatings for food and confectionery products. Plasticizers were added at typical levels after correction of base film-forming material for moisture content. Surfactants, which must be added to water-based coatings to ensure proper wetting and adhesion of coating, may influence gloss. Lipids, which are added to improve the moisture resistance to WP coatings, may also change gloss.

### Coating liquid preparation

**Whey protein coatings.** Ten percent whey protein coating liquids were made from WPI (Bipro<sup>®</sup>, Davisco Foods International, LeSuer, Minn., U.S.A.) or WPC (AMP<sup>®</sup> 800, AMPC Inc., Ames, Iowa, U.S.A.) powder in distilled water, denaturing the protein by heating the liquids for 20 min at 90 °C, and cooling in an ice bath to 25 °C with constant agitation. Solutions were then equilibrated to room temperature, at which point glycerol (USP/FCC, Fisher Scientific, Fairlawn, N.J., U.S.A.) was added as a plasticizer to a level of 1.5 g of protein powder to every 1 g of glycerol, on a dry solids basis. Dry powder composition for WPI was 5.3% moisture, 93% protein, < 0.01% fat, < 0.1% lactose, and 1.6% ash; while WPC composition was 5.5% moisture,

80% protein, 5.7% fat, 3.9% lactose, and 4.9% ash. Analyses were performed by Silliker/DFL Labs (Modesto, Calif., U.S.A.) and the University of California, Davis, Division of Agriculture and Natural Resources (DANR) analytical laboratory. WPC formulations were opaque, pale yellow dispersions. WPI formed translucent, slightly yellow solutions. Final solids content of whey protein coating solutions was 13.7% (wt/wt).

Dispersion coatings were made by adding carnauba wax (CW) (Strahl and Pitsch, West Babylon, N.Y., U.S.A.) or anhydrous milkfat (AMF) (Level Valley Dairy, West Bend, Wis., U.S.A.) to whey protein formulations. Aqueous mixtures of WPI, glycerol, and a lipid were heated and maintained at 90 °C to ensure carnauba wax (melting point of 83 to 86 °C) and AMF (melting point of 35 to 40 °C) remained liquid during homogenization. WPI-CW dispersions (40% wax on a dry solids basis) were made using a Microfluidizer homogenizer (HC 5000, Microfluidics International Corp., Newbury, Mass., U.S.A.). The hot liquid was passed through the homogenizer 3 times using homogenizing pressure of 6000 psig. Anhydrous milk fat emulsions (40% fat on a dry solids basis) were made using a Crepac homogenizer (Crepaco Inc., Chicago, Ill., U.S.A.), with 3 passes at 8500 psig.

**Shellac.** Dewaxed, bleached shellac (Type R-49, Mantrose-Haeuser Co., Attleboro, Mass., U.S.A.) was dissolved in 95% ethanol to form 29% (wt/wt) solutions. Propylene glycol (USP/

FCC, Texaco Chemical Co., Houston, Tex., U.S.A.) was added at level of 1 part propylene glycol to 9 parts shellac. The mixture was stirred until shellac was completely dissolved and then strained through 2 layers of cheese cloth. The resulting solution had solids content of 32% (wt/wt).

Shellac-carnauba wax dispersions were made by milling a 47.6% (dry solids basis) dispersion of powdered carnauba wax in the above shellac solutions using a stone-type media mill (Norton, Plastics and Synthetics Division, Lab Equipment Products, Akron, Ohio, U.S.A.). Wax particle size became constant after about 96 h of milling.

**Dextrin.** Twenty percent tapioca dextrin (K-4484, National Starch and Chemical Co., Bridgewater, N.J., U.S.A.) solutions were prepared by dissolving the powder in hot (40 °C) water and mixing for 20 min. Propylene glycol was then added at a 1 part propylene glycol to 6 parts dextrin. Solutions were cooled by mixing without heat until room temperature (25 °C) was reached. Dextrin solutions had final solids levels of 24% (wt/wt).

**Zein.** Fourteen percent zein coating solutions were prepared by dissolving zein (FC 4000, Freeman Industries, Tuckahoe, N.Y., U.S.A.) in 95% ethanol. Propylene glycol was then added to a level of 1 part propylene glycol to 3 parts zein. Final zein solutions contained 20% (wt/wt) of solids.

**HPMC.** Five percent (wt/wt) solutions of HPMC (Methocel E5 Premium, Dow Chemical Co., Midland, Mich., U.S.A.) were prepared according to the manufacturer's instructions. The process consisted of dispersing HPMC powder into 1/3 of the total required amount of water at 85°C, followed by gentle stirring until the powder was wetted and a consistent dispersion was obtained. The remaining 2/3 of water was then added and the dispersion was mixed until it became a clear solution. The solution was then removed from the heat and mixed until it equilibrated to room temperature. Finally, glycerol was added to a 5:1 (wt/wt) ratio of HPMC to glycerol. Final HPMC solutions contained 5.6% (wt/wt) of solids.

### Surfactant addition

Three different surfactants, sorbitan monolaurate (Span 20, Sigma Chemical, St. Louis, Mo., U.S.A.), polyoxyethylene sorbitan trioleate (Tween 85, Sigma Chemical, St. Louis, Mo., U.S.A.) and lecithin (Centrolene® A, Central Soya Company, Fort Wayne, Ind., U.S.A.) were added to achieve levels of about 0.1% to 4% on a dry solids basis. Tween 85 has a hydrophilic-lyophilic balance (HLB) value of 11. Span 20, which is more hydrophobic than Tween 85, has an HLB value of 8.6 (Ash and Ash 1981). Centrolene® A is a charged surfactant blend of a number of phospholipids and, strictly speaking, does not have an HLB number. However, the manufacturer reports that Centrolene® A has an approximate HLB value of 10 (Anon 1989). This number can be used as a guide when one uses a lecithin in place of a synthetic, nonionic surfactant.

### Coating casting and drying

Liquid coating formulations were cast on 4 in × 7 in × 1/8 in thick sheets of matteblack acrylic plastic (TAP Plastics, Sacramento, Calif., U.S.A.), using a Bird-type applicator (Paul Gardner Co., Pompano Beach, Fla., U.S.A.). Matte black (low gloss) was used to avoid double reflection that might occur from a high gloss surface. Coatings were then dried at ambient conditions (23 to 25 °C, 35% to 45% RH) for 16 h. Dry coating thickness was 25 μm.

### Particle size analysis

Dispersion particle size analyses were made using a Malvern MS 20 particle size analyzer (Malvern Instruments, Malvern, England). Parameters chosen for operation included a lens focal length of 45 mm and an obscuration value maintained to be between 0.14 to 0.30 (within the instrument ideal range). The background-zeroing liquids used were 95% ethanol and distilled water for shellac and WPI dispersions, respectively. A presentation code of 0409 was used in the measurements, which refers to a refractive index ratio (dispersed phase/continuous phase) of 1.08 and an extinction index (k) of 0.01. This k value for carnauba wax and milkfat was recommended by scientists at Malvern. The SMD was used as the characteristic dimension of the particle size. The SMD is defined as the ratio of the 3<sup>rd</sup> to 2<sup>nd</sup> moments of the probability density function of the dispersed particles (Pacek and others 1998). It is also called the volume-surface mean, which is the average size of dispersed particles based on the specific volume per unit surface (Stockham 1977). The SMD is commonly noted as  $D[3,2]$  or  $d_{32}$ . Other important parameters include  $D[v,0.9]$ ,  $D[v,0.5]$ , the span and the shape of the size distribution.  $D[v,0.9]$  and  $D[v,0.5]$  specify the mean particle diameter at the 90<sup>th</sup> and 50<sup>th</sup> percentiles, respectively (Malvern, year unknown). The span gives a measure of the width of the volume distribution relative to the median diameter ( $D[v,0.5]$ ), and is calculated as  $(D[v,0.9] - D[v,0.1])/D[v,0.5]$ .

### Gloss measurements and haze calculations

Gloss was measured using a MICRO-TRI-GLOSS meter (BYK Gardner, Silver Spring, Md., U.S.A.). The gloss is the intensity of light that is specularly reflected from a sample surface compared to the intensity of light that is specularly reflected from a known standard surface at a given angle of incidence (ASTM 1995a). In this case, a polished black glass plate with a refractive index of 1.567 was used as the standard surface (ASTM 1995a). The gloss is generally expressed in percent units, with the standard having a gloss of 100 at each angle of incidence. Gloss was measured at 20 °, 60 °, and 85 ° angles from the normal to the coating surface (ASTM 1995a). A surface with a 60 ° gloss of 70 or greater is considered a high gloss surface (BYK 1997). The 20 ° angle can then help to differentiate between 2 high gloss samples; whereas the 85 ° angle differentiates between low gloss samples (when 60 ° gloss is less than 10) (BYK 1997). The gloss values of the matte black casting surfaces were about 0.5, 3, and 12 for the 20 °, 60 ° and 85 ° measurement angles, respectively.

Haze Indexes were calculated according to ASTM method D4039 (ASTM 1995b) as  $G^{60}-G^{20}$ . The values  $G^{60}$  and  $G^{20}$  are the gloss measured at an angle of incidence of 60 ° and 20 °, respectively. Increasing Haze Index values indicate increasing surface irregularity. A Haze Index of zero would mean that the surface is perfectly reflecting.

### Relative humidity storage

Coatings were stored in environmental chambers (Fisher Scientific, Fairlawn, N.J., U.S.A.) at RHs of 23%, 52%, 75%, and 95%. RH was maintained in the chambers by saturated salt solutions of potassium acetate (23%), magnesium nitrate (52%), and sodium chloride (75%) (Carr and Harris 1949; Rockland 1960; Young 1967.) Distilled water was used to maintain 95% RH environments. The chambers were stored at 23 °C inside a controlled temperature room. To ensure uniform conditions at

the surface of each film, fans inside each chamber produced a minimum airflow of 152 ft·min<sup>-1</sup> (McHugh and others 1993). Pen-style thermohygrometers (Fisher Scientific, Fairlawn, N.J., U.S.A.) were used to monitor temperature and RH inside the environmental chambers.

### Whiteness index of films

Some of the films "blushed," (became white), as storage RH was increased. Whiteness index of the coatings was measured using a Hunter LabScan colorimeter (HunterLab, Reston, Va., U.S.A.). Four measurements of the L, a, b parameters were measured across each film. Instrument parameters were Mode, 0/45; Observer, 2 °; illuminant, D65 sodium; port size,

0.25 in and area view, 0.25 in. Whiteness index (WI) was calculated according to the equation used by Cisneros-Zevallos and others (1997).

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{0.5} \quad (1)$$

### Statistical analysis

Experiments conducted were completely randomized designs. For all samples a minimum of three replicates were prepared. Statistical analyses were performed using analysis of variance and the Fisher LSD multiple comparison test using StatView software (Version 4.5, Abacus Concepts Inc., Berkeley, Calif., U.S.A.).

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