

## Influence of Emulsifier Type and Content on Functional Properties of Polysaccharide Lipid-Based Edible Films

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This study investigates the effect of different types of surfactant (glycerol monostearate, Tween 60, and Tween 80) on water vapor permeability (WVP), tensile strength (TS), percentage elongation at breaking ( $E$ ), and structure of an emulsified edible film composed of cornstarch, methylcellulose, and cocoa butter or soybean oil. Factorial designs at two levels were used to analyze the effect of emulsifier (EM) and lipid content on the functional properties of film. Results showed that the effects of independent variables on WVP, TS, and  $E$  depend on surfactant and lipid type. The presence of EM significantly decreased the WVP of cocoa butter films but did not improve the barrier or mechanical properties of soybean oil-based film.

**KEYWORDS:** Emulsified edible film; surfactant; water vapor permeability; soybean oil; cocoa butter

### INTRODUCTION

Materials used to make edible films include polysaccharides, proteins, and lipids or combinations of these (1). Composite films make it possible to combine the advantages associated with different components. Lipids can create effective barriers to water vapor, and hydrocolloids provide structural support, as well as limit oxygen and carbon dioxide transfer (1–3).

Starch is a natural polymer that can readily be cast into films. The interest in starch, and other polymers of vegetal origin, stems from their inherent biodegradability, availability, and low cost (4). Starch films have poor physical properties, but these can be improved by blending starch with cellulose derivatives and proteins (5–8).

Cellulose and its derivatives such as ethers and esters are, with starch, the most important raw materials for the preparation of films. The usefulness of cellulose as a material for edible films may be extended by chemical modification. One such modified version is methylcellulose (MC), a water-soluble ether with good film-forming properties. Glycerol and poly(ethylene glycol)s have been shown to be the most effective plasticizers for MC (9, 10). Cellulose-based films are efficient oxygen and aroma barriers, but they are ineffective against water vapor because they are hydrophilic (10, 11). The water vapor barrier properties of cellulose-based films were improved by the addition of lipids (12, 13). Generally, the water vapor permeability (WVP) of a composite polymer lipid-based edible film increases as the length of the lipid hydrocarbon chain decreases and as unsaturation, or branching of acyl chains, increases as a result of enhanced mobility and less efficient packing of chains (13, 14). Hydrophobic lipids, such as alkanes and waxes, have the best water vapor barrier efficiency. The presence of polar groups decreases the resistance to water transfer of more

hydrophilic lipids (15–17). Several authors showed the influence of lipid physical state and polymorphism on the functional properties of composite edible films (18–20).

Lipids can be dispersed in hydrocolloid aqueous solution and dried to obtain an emulsified film or to form a layer on the hydrocolloid film used as a mechanical support, thus obtaining a bilayer film. Emulsion-based films are not as effective a barrier as bilayer films because the dispersed lipid phase only modifies apparent tortuosity, and water vapor can migrate through the continuous hydrophilic matrix (14, 21). Despite these characteristics, the food industry has focused its research on emulsified films, which require only one step in manufacture, as opposed to the three steps required for bilayer films. Barrier properties of emulsion film are influenced by particle size and lipid globule distribution. McHugh and Krochta (22) observed that a decrease in mean particle lipid diameters of protein–beeswax film-forming emulsion correlated well with a linear decrease in film WVP. This was also noted by Pérez-Gago and Krochta (23), but only at high lipid content. At low lipid content, the hydrophilic protein matrix still dominates in the final composition of the film.

The effect of the drying temperature of the film-forming emulsion on the WVP of composite films has been attributed to several factors, including cohesion within the film polymer matrix, and change in polymer arrangement and lipid morphology, as well as change in lipid distribution (24–26). Temperature increase may lower the viscosity of the continuous phase and melt fat, which would permit coalescence and phase separation, giving the final film an apparent bilayer structure. Pérez-Gago and Krochta (23) demonstrated that film from an unstable emulsion showing some phase separation did not give a better film barrier than film from a stable emulsion.

Emulsifiers may stabilize film forming during drying, but only a few papers have studied the effect of added emulsifier on composite film functional properties. Debeaufort and Voilley

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**Table 1.** Experimental Design and Levels of Independent Variables

design point	coded value independent variables		exptl design independent variables	
	$X_1$	$X_2$	$L^a$ (%)	$EM^b$ (%)
1	-1	-1	10	10
2	-1	+1	10	30
3	+1	-1	20	10
4	+1	+1	20	30

<sup>a</sup> On dry matter. <sup>b</sup> On total lipid.

(24) observed that emulsifiers reduce the WVP of MC–paraffin wax films. The authors noted that emulsifying efficiency depended mainly on the chemical nature of the surfactant and less on its hydrophobic or hydrophilic properties.

Sucroesters contribute to the formation of smaller lipid globules in arabinoxylan–hydrogenated oil emulsion and stabilize the emulsion itself (27). The moisture barrier performance of film-forming emulsions is improved by the homogeneous distribution of small lipid globules within the film and the significant structural stability of the emulsion during drying or by the significant destabilization of the emulsion by creaming, aggregation, and/or coalescence of the lipids at the evaporating surface.

In our previous works (8, 28), a starch–MC edible film was developed by evaluating and optimizing the combined effects of plasticizer (glycerol) content and blending levels of methylcellulose (MC) with starch on film properties, using response surface methodology. The objective of this work was to investigate the influence of the type and concentration of emulsifiers and lipids on the functional properties and structure of optimized starch–MC edible film.

## MATERIALS AND METHODS

**Materials.** Corn starch (27% amylose, Sigma) and methylcellulose (MC, medium viscosity, 27.5–32% methoxyl content, Fluka) were used as film-forming components of the hydrophilic continuous phase for emulsion-based edible films; commercial soybean oil or cocoa butter (Callebaut) was used as the hydrophobic disperse phase, and glycerol (Baker) was added as plasticizer. Glycerol monostearate (GMS, Prolabo), polyoxyethylene sorbitanmonostearate (Tween 60), and polyoxyethylene sorbitanmonooleate (Tween 80) from Sigma were tested as emulsifiers to improve film-forming emulsion stability. Ethyl alcohol and magnesium nitrate were purchased from Fluka.

**Experimental Design.** A two-factor, two-level experimental design was chosen to study the effects of total lipid content (L) and emulsifier content (EM) on edible film properties. Total lipid content (10 and 20% on dry matter as starch plus MC plus glycerol plus L) and emulsifier content (10 and 30% on total lipid content as oil or cocoa butter plus EM) were established according to literature data and preliminary tests. The two levels of each variable were coded -1 and +1. **Table 1** presents the experimental design selected, which consists of four design points, each performed in triplicate for a total of 12 samples. Three different emulsifiers (GMS, Tween 60, and Tween 80) and two types of lipids (soybean oil and cocoa butter) were tested. A total of six different experimental designs were performed to investigate combinations of different emulsifier and lipid types. The sequence of experiments was fully randomized to avoid systematic errors. Homogeneous films without emulsifier were used as reference. Dependent variables were water vapor permeability and mechanical properties.

**Preparation of Emulsified Films.** Edible film-forming dispersions were obtained by dispersion and solubilization of MC (1.44 g) in 75 mL of a distilled water/ethyl alcohol mixture (2:1, v/v) at 75 °C for 10 min and by dispersion and gelatinization of corn starch (3.19 g) in 75 mL of water at 95 °C for 30 min. Gelatinized starch was homogenized at 4000 rpm for 1 min (Polytron PT 3000, Kinematica AG). Subsequently, glycerol (1.16 g) was added to the MC, and the solution was

homogenized at 6000 rpm for 1 min. The components were mixed with a magnetic stirrer (800 rpm). MC and starch preparations were mixed together, homogenized at 6000 rpm for 90 s, and maintained at 75 °C for 10 min under stirring. The addition of lipid (soybean oil or molten cocoa butter at 75 °C) and emulsifier (GMS, Tween 60, and Tween 80) differed according to the hydrophilic lipophilic balances (HLB) of the emulsifier. For Tween 60 and Tween 80 (hydrophilic), the lipid and the emulsifier were successively added to the starch–MC–glycerol dispersion as reported by Phan The et al. (27). For GMS (hydrophobic), the emulsifier was first solubilized at 75 °C in the oil or molten cocoa butter, prior to being added to the starch–MC–glycerol dispersion (27). After the addition of lipid and emulsifier, the mixture was predispersed under magnetic stirring for 2 min at 800 rpm and 75 °C, before being homogenized at 6000 rpm for 2 min. The emulsion was then maintained under magnetic stirring for 10 min at 800 rpm and 75 °C, before being spread onto glass plates (25 × 50 × 1 cm) with a thin-layer chromatography spreader. Films were dried at 25 °C, at a relative humidity of ~40%, for 15 h. Film thickness was  $30 \pm 3 \mu\text{m}$  (Elcometer 345 digital micrometer, Elcometer Instruments). Before measurements, films were maintained at a constant relative humidity of 53% and a temperature of 25 °C for 10 days, using a saturated magnesium nitrate solution.

**Water Vapor Permeability.** Water vapor transfer rate (WVTR) was measured gravimetrically at a constant difference of relative humidity (RH), and at 25 °C, using a modification of ASTM standard method E 96-80 (29). The test film was sealed to a glass permeation cell containing distilled water, and the cell was placed in a climatic room maintained at 22% RH, with a saturated salt solution of potassium acetate (Sigma). The climatic room was equipped with fans to eliminate stagnant air above the test cell. Cell weight was recorded periodically on a computer to evaluate stationary-state water vapor transfer. WVTR ( $\text{g m}^{-2} \text{s}^{-1}$ ) and WVP ( $\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$ ) were calculated using the equations

$$\text{WVTR} = \frac{\Delta m}{A \Delta t} \quad (1)$$

$$\text{WVP} = \frac{\text{WVTR} \times x}{p_1 - p_2} \quad (2)$$

where  $\Delta m$  is weight loss of the permeation cell, with  $4.9 \times 10^{-4} \text{ m}^2$  exposed area ( $A$ ), over time ( $\Delta t$ ),  $x$  is film thickness, and  $p_1 - p_2$  is real vapor partial pressure difference (Pa) across the film. The real vapor partial pressure at the film inner surface ( $p_1$ ) was corrected for the stagnant air gap inside the test cell, according to the method of Gennadios et al. (30). After this correction, the difference in relative humidity varied between 22 and 82% and between 22 and 88%.

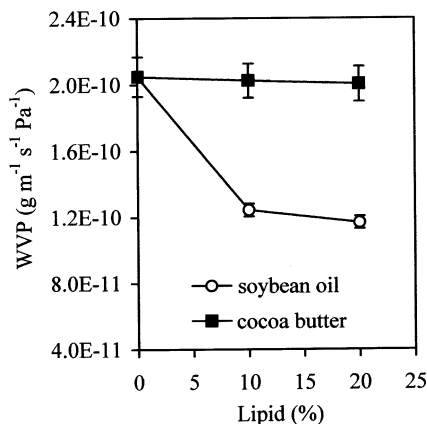
Few authors (15) use water vapor permeance expression ( $\text{g s}^{-1} \text{m}^{-2} \text{Pa}^{-1}$ ) to describe water vapor transmission through composite films, where

$$\text{WVP} = \text{permeance} \times x \quad (3)$$

Use of the standard permeability (WVP) for heterogeneous barriers could be considered inappropriate because total film thickness does not accurately reflect the barrier properties of the individual film component. Because the film thickness is consistent among samples being compared, permeability and permeance show the same differences on film water vapor barrier properties. Data of this experimental work were expressed as WVP.

**Mechanical Properties.** Tensile strength (TS, MPa) and percentage elongation ( $E$ , %) at breakpoint were measured uniaxially by stretching the specimen (10 × 2.5 cm) in one direction at 50 mm/min using an Instron Universal Testing Instrument (model 4301). The films were analyzed in a climatic room at 53% RH and 25 °C. Initial grip separation was set at 4 cm. TS was calculated by dividing the maximum load by the cross-sectional area of the film,  $E$  being expressed as a percentage of change in the original specimen length between grips (4 cm), according to ASTM standard method D 882-88 (31).

**Environmental Scanning Electron Microscopy (ESEM).** Film cross section was observed using ESEM (model XL 30, Phillips). Small



**Figure 1.** Water vapor permeability (WVP) of emulsion films as a function of lipid contents.

film strips (5 × 3 mm) were fixed on the support using double-sided adhesive tape. An accelerating voltage of 15 kV was used.

**Statistical Analysis of Data.** WVP results are reported as the mean of 12 measurements from three different preparations. Data referring to mechanical properties are expressed as the mean of 21 measurements from three different preparations for all of the formulations.

Two-way analysis of variance (ANOVA) was conducted to determine the effects of L and EM on film properties for each experimental design (Statistica software version 5, 1997). When interaction was not significant, Student's *t* test was performed on the main factors to determine significant differences between means ( $p < 0.05$ ).

When interaction was significant, data were analyzed by L or EM content, at constant EM or L content, respectively. Student's *t* test was then performed.

The effect of EM type was investigated using Duncan's multiple-comparison test to determine significant differences between means.

## RESULTS AND DISCUSSION

**Effect of Lipid Type and Content on Water Vapor Permeability.** Figure 1 shows the effect of adding increasing quantities of lipids on the WVP of a hydrophilic edible film containing previously optimized starch–MC–glycerol (8, 28). The addition of 10% oil determined a significant reduction of WVP with respect to the lipid-free reference, as observed by other authors (32). Water vapor barrier properties are reported to be improved by the presence of hydrophobic-type physical obstacles, which are believed to hinder the transfer of water molecules inside the film (16, 33, 34). Nevertheless, a proportion of oil in excess of 10% did not contribute to any further improvement in edible film barrier properties, because the negative effect of the polar groups can be only partially reduced by the presence of apolar compounds (16, 35). An increase in WVP was observed by Sapru and Labuza (36) for quantities of stearic acid above a specific critical value.

Formulations containing cocoa butter presented no significant differences in WVP with respect to the reference, as observed by Handa et al. (37) for proteic films with added milk lipid fractions (Figure 1). This result apparently contradicts the observations of other authors, who registered an increase in the barrier properties in the presence of high melting point lipids with high quantities of solid fraction (19, 35, 38).

However, an investigation of lipid fractions comprising a solid phase mixed with a liquid phase showed an increase in WVP beyond a certain quantity of solid-state apolar components. This is caused by the presence of zones of discontinuity that, it is argued, facilitate the transfer of water molecules inside the film (19).

Images of film sections containing 20% lipids revealed, in the presence of oil, a compact lamellar structure (Figure 2a,b) comprising lipid-covered starch and MC fibers. In contrast, the addition of butter determined the formation of a less oriented, less compact structure with evident internal cavities and fat crystals on the surface (Figure 2c,d). Cocoa butter added to the starch–MC–glycerol formulation therefore appears to be ineffective as a barrier to water vapor because of the formation of crystallization-related discontinuities. The superior performance of oil may be attributable to its liquid state, which could promote continuity by increasing resistance to the transfer of water molecules.

The WVP of an edible film containing starch and MC may be significantly reduced by the addition of soybean oil in a proportion of 10–20%, but cocoa butter has no effect. For this reason, we proceeded to study the addition to the formulation of emulsifiers that might improve the functional properties of the film.

**Effect of Lipids and Emulsifiers on Water Vapor Permeability.** Table 2 shows results from the analysis of the effects of lipids (L), emulsifiers (EM), and their interaction (L–EM) on WVP of edible films containing cocoa butter or soybean oil. No significant effects of L, EM, or L–EM on WVP were observed for GMS samples or for Tween 60–soybean oil, whereas lipid content was significant for Tween 60–cocoa butter film. When Tween 80 was used, L–EM had an effect on WVP. L and EM were significant for the cocoa butter formulation, but these effects are not considered because evidence of interaction implies that the interacting variables have to be considered jointly.

WVP values of films in relation to lipid and emulsifier content are reported in Table 3. WVP ranged from  $(13.31 \pm 0.21) \times 10^{-11}$  to  $(20.99 \pm 1.18) \times 10^{-11}$  g m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup> in different samples. These results fall within the range for other emulsified edible films. WVP values of  $13.20 \times 10^{-11}$  g m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup> were reported for MC–hydrogenated palm oil (39). Synthetic films, such as cellophane and LDPE, gave WVP values of  $8.4 \times 10^{-11}$  and  $0.02 \times 10^{-11}$  g m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup>, respectively (34).

Tween 60–cocoa butter films showed lower WVP at higher lipid content (Table 3). Increased Tween 80 content resulted in lower WVP for edible films containing 10% lipids, whereas no significant differences were observed at higher lipid content (Table 3). For Tween 80–soybean oil formulations, water vapor barrier properties did not change significantly when soybean oil was increased from 10 to 20% at low emulsifier content. At higher EM, WVP rose as soybean oil increased. Moreover, a substantial reduction in WVP was observed with increasing cocoa butter for samples at 10% Tween 80. No benefit was observed when Tween 80 content was increased in formulations prepared using 20% cocoa butter.

Results showed that the effects of L and EM on barrier properties depend primarily on the nature of the emulsifier (24). GMS and Tweens are nonionic, or uncharged, low molecular weight surfactants with different hydrophilic lipophilic balances (HLB). GMS is hydrophobic (HLB = 4–5) and Tweens are hydrophilic (HLB = 14–15). Tween 60 and Tween 80 exhibit the same hydrophilic group and number of carbon atoms in the hydrophobic chain, but Tween 80 is monounsaturated as it contains a double bond, which implies a certain degree of molecular flexibility. The emulsifier should stabilize the dispersed lipid droplets, preventing them from flocculating or coalescing during film preparation and drying. Water vapor barrier properties depend primarily on the distribution of lipid globules within the starch–MC matrix. Homogeneous distribu-

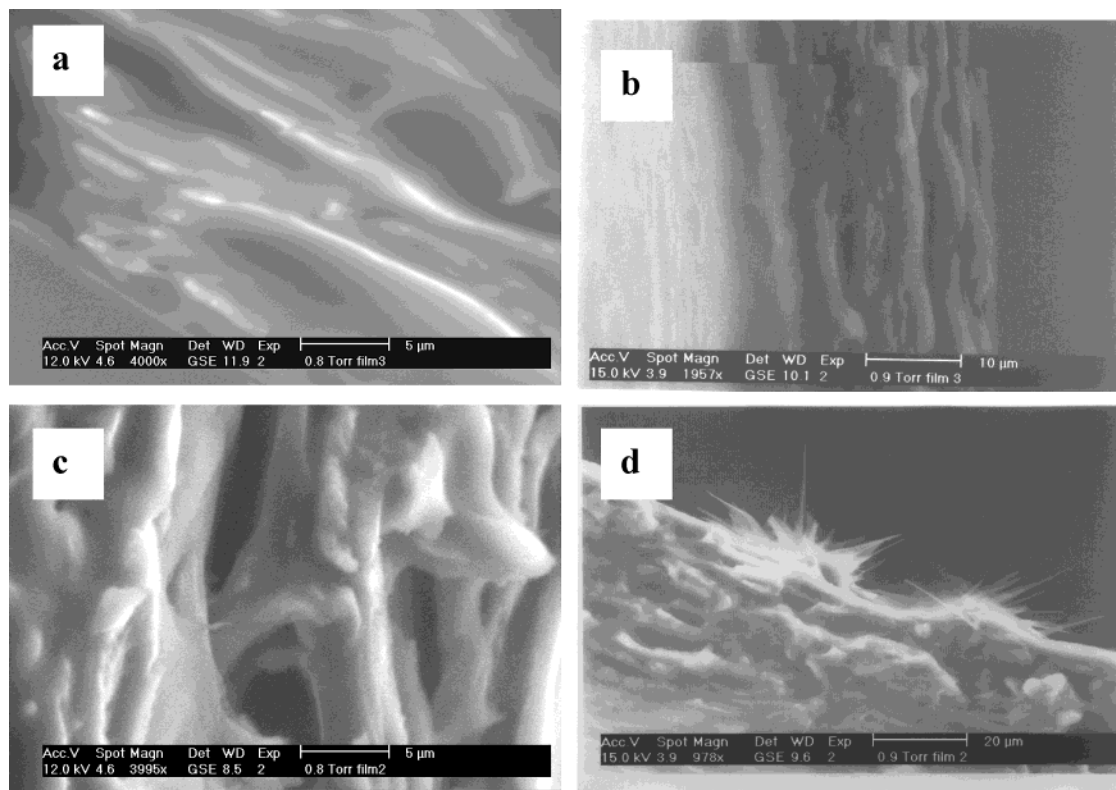


Figure 2. ESEM cross-section micrographs of 10% soybean oil films (a, b) and 20% cocoa butter films (c, d).

Table 2. Variance (*F* Value) of Lipid Content (L) and Emulsifier Content (EM) on Water Vapor Permeability (WVP) of Edible Film Containing Cocoa Butter or Soybean Oil, Related to Emulsifier Type (GMS, Tween 60, and Tween 80)

surfactant	source	<i>F</i> value <sup>a</sup>	
		soybean oil WVP (g m <sup>-1</sup> s <sup>-1</sup> Pa <sup>-1</sup> )	cocoa butter WVP (g m <sup>-1</sup> s <sup>-1</sup> Pa <sup>-1</sup> )
GMS	L	1.49	0.73
	EM	0.22	0.15
	interaction L-EM	1.40	0.56
Tween 60	L	2.08	16.03*
	EM	2.03	1.89
	interaction L-EM	1.05	2.97
Tween 80	L	1.21	19.04*
	EM	4.83	14.41*
	interaction L-EM	10.63*	9.58*

<sup>a</sup>\*, significant at  $p \leq 0.05$ .

tion of lipids and small-diameter globules decrease water vapor transfer through emulsified films (22–24, 27). Large globules produce heterogeneous structures containing regions with no hydrophobic compounds, which are inefficient for moisture transfer control.

Hydrophobic GMS did not exhibit any significant effect on WVP of our films, probably because of low solubility in the continuous hydrophilic phase of the film-forming emulsion (Tables 2 and 3). Tween 80, which is fully soluble in water, was most effective, but a similar behavior was not observed for the hydrophilic Tween 60. Water vapor transfer cannot therefore be explained by HLB alone (16, 24, 27). Different behaviors depend mainly on the nature and concentration of the emulsifier (27). Films based on Tween 80 showed a strong influence of emulsifier content on WVP at 10% lipid, but this behavior changed at higher lipid content, demonstrating that

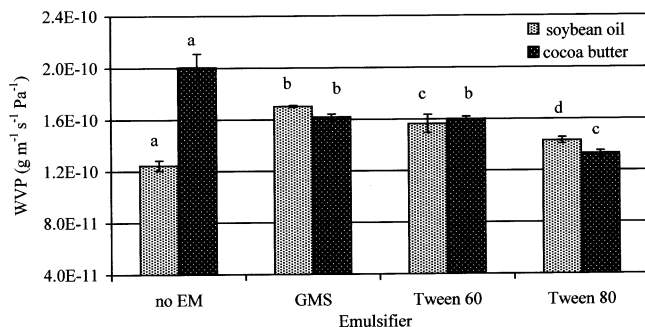
Table 3. Water Vapor Permeability<sup>a</sup> (WVP,  $\times 10^{-11}$  g m<sup>-1</sup> s<sup>-1</sup> Pa<sup>-1</sup>) of Edible Film Containing Cocoa Butter or Soybean Oil Related to Lipid Content (L), Emulsifier Content (EM), and Emulsifier Type (GMS, Tween 60, and Tween 80)

	EM (%)	L (%)	
		10	20
Soybean Oil			
GMS	10	15.71 ± 1.13	15.68 ± 1.67
	30	17.10 ± 0.90	15.10 ± 1.05
Tween 60	10	15.89 ± 1.09	17.37 ± 0.71
	30	15.65 ± 0.71	15.90 ± 0.83
Tween 80	10	18.75 ± 1.46 Aa	16.93 ± 0.91 Aa
	30	14.16 ± 0.50 Ba	17.83 ± 1.57 Ab
Cocoa Butter			
GMS	10	18.15 ± 1.97	17.24 ± 0.97
	30	15.95 ± 1.36	16.19 ± 0.23
Tween 60	10	18.11 ± 0.93	15.87 ± 0.55
	30	16.90 ± 0.03	16.01 ± 0.18
	mean	17.50 a	15.94 b
Tween 80	10	20.99 ± 1.18 Aa	13.97 ± 1.77 Ab
	30	14.51 ± 1.59 Ba	13.31 ± 0.21 Aa

<sup>a</sup> Means ± standard deviations. Means with the same capital letter in a column are not significantly different ( $p < 0.05$ ); means with the same small letter in a row are not significantly different ( $p < 0.05$ ).

water barrier properties depend on the EM–L interaction and lipid type (Table 2) (40).

Figure 3 shows the WVP of 20% cocoa butter films containing 30% EM and no EM and 10% soybean oil films with no EM and 30% EM to assess if EM addition is beneficial. Films containing EM were selected on the basis of the best water vapor barrier properties (Table 3). Of the various emulsifiers, Tween 80 gave the lowest WVP. Water vapor barrier properties of soybean oil films containing EM were inferior to those shown



**Figure 3.** Water vapor permeability (WVP) of 20% cocoa butter films containing no EM and 30% EM and of 10% soybean oil films with no EM and 30% EM. Values are means, and standard deviations are represented by bars. Means with the same letters are not significantly different ( $p < 0.05$ ); statistical analyses are made independently for oil and butter.

by the same formulations without EM. WVP for cocoa butter films was lower in the presence of emulsifiers.

ESEM observations of film cross sections were used to investigate the component-dependent structure of the films (Figures 2 and 4). Cocoa butter films without EM showed cavities within the matrix and cocoa butter crystals on the evaporation surface (Figure 2). In contrast, film with GMS and Tween 60 presented compact structure (Figure 4a,b). Some authors (24, 27) reported that emulsifiers contributed to a more homogeneous distribution of smaller lipid globules in the film matrix. Formation of large crystallites during and after film drying within the MC–starch continuous layer may give rise to defects, through which water vapor can pass (36, 41). GMS and Tween 60 seem to reduce WVP, preventing defects and, probably, improving homogeneity of butter distribution within the film. The smaller the lipid globule size, and the more homogeneously the globules are distributed, the lower the WVP (22, 24). In emulsified films, water migrates preferentially through the continuous hydrophilic matrix, and the dispersed lipid phase only modifies the apparent tortuosity.

Films based on Tween 80 had a lower WVP than those from GMS or Tween 60 (Figure 3). As illustrated in Figure 4c, film prepared with Tween 80 exhibited a compact structure, but fat was not homogeneously distributed. Individual crystals could be discerned on the surface, and fat bloom was visually observed. Lipid creaming toward the evaporation surface contributed to a decrease in permeability because the structure tended to resemble a bilayer film (26, 39). In the absence of EM, lipid phase separation did not improve vapor barrier properties because of the film's porous structure. Before measurement, films were stored in the same conditions, and for the same length of time, so the results show that GMS and Tween 60 prevented an evident fat separation within the films, whereas Tween 80 did not.

Tweens may influence the rate of polymorphic transformation in cocoa butter (42), which significantly affects film permeability (18, 41). The addition of EM to cocoa butter may affect the rate of polymorphic transformation, and the segregation of high-melting fractions from lower fractions. This may be especially significant in relation to the problem of fat bloom (43). Tween 60 and Tween 80 exhibited the same effect on polymorphism of cocoa butter in bulk (42), but for *n*-hexadecane oil-in-water emulsions, the crystallization temperature differed for the two emulsifiers (44). The crystallization of fat in bulk proceeds quite differently from that of the same fat in a dispersed or emulsified form (45), so it is reasonable to suppose that the differences observed in the barrier properties of films containing Tween

60 and Tween 80 may well be linked to different crystallizations of cocoa butter (44).

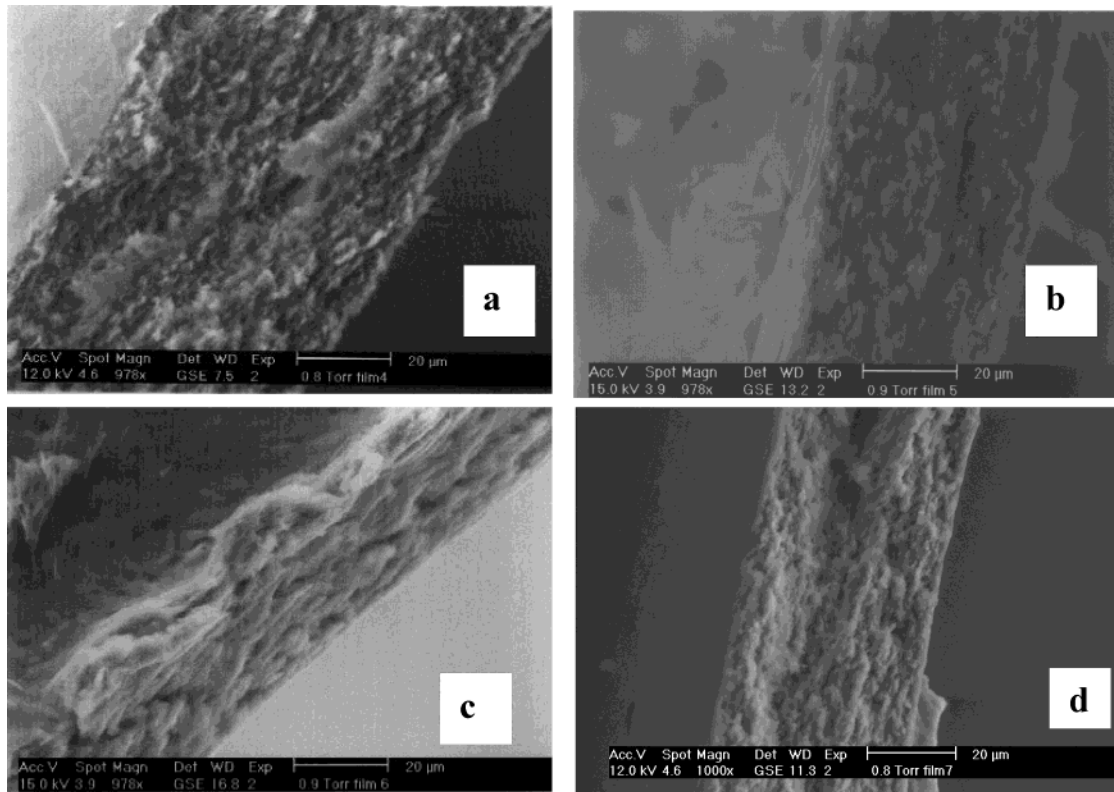
ESEM micrographs of the film cross section showed that oil–film without emulsifiers exists as multilaminar packed layers of starch and MC, probably covered with oil, and that these layers are arranged perpendicular to the direction of vapor flow (Figure 2). Oil–GMS film did not show a multilayer arrangement and presented a compact structure (Figure 4d). Cross sections of oil–Tween 60 and oil–Tween 80 films were the same as those of oil–GMS films (images not shown). In our opinion, EM did not improve film structure. The most significant effect of EM was to increase the polarity of the lipid phase, promoting water molecule sorption and water vapor mobility (1, 16, 33, 39).

**Effect of Cocoa Butter and Emulsifiers on Mechanical Properties.** For a film formed on the surface of a food to be used successfully, the film must have some degree of structural integrity. Loss of structural integrity due to poor mechanical properties reduces the film's effectiveness as a barrier.

Table 4 shows the results from the analysis of effects of lipid and emulsifier content, and their interactions, on tensile properties of cocoa butter films. Lipid content had a significant effect on tensile strength for GMS and Tween 60 films, whereas Tween 80 showed an interaction effect. When Tween 60 was used, EM had an effect on TS. No significant effects of L and EM on elongation were observed for Tween 80 samples, but interaction had an effect on E when GMS and Tween 60 were used (Table 4). Table 5 shows TS and E values of cocoa butter films in relation to lipid and emulsifier contents. Mechanical properties showed substantial variations, depending on film formulation. Tensile strength ranged from  $6.66 \pm 0.30$  to  $20.64 \pm 1.17$  MPa, and elongation varied from  $3.70 \pm 0.19$  to  $28.63 \pm 3.15\%$  (Table 5). TS values are in the same range as for low-density polyethylene film (8.3–27.6 MPa) (7, 10). According to the literature, an increasing total lipid level reduced the TS of all composite films (23, 34). The effect of L on TS for Tween 80 film was higher at low emulsifier concentration than at high concentration because of L–EM interaction (Table 5). Lipid addition induces development of a heterogeneous film structure featuring discontinuities in the polymer network. The result is a decrease in TS because of preferential break points (25, 34). These discontinuities may enhance film brittleness as L increases (smaller deformation to breaking), as observed for samples prepared with 10% GMS and 10% Tween 60 (Table 5).

Increased GMS and Tween 80 content resulted in lower E for edible films containing 10% lipids, whereas no significant differences were observed at higher lipid content (Table 5). Tween 80 gave the lowest E and the highest TS values with different emulsifiers for formulations at 10% lipids, whereas GMS and Tween 60 samples exhibited similar mechanical properties. It seems that Tween 80 enhanced cocoa butter–polysaccharide interaction, or polysaccharide–polysaccharide interaction, more than Tween 60 and GMS. These results confirm that there was no direct correlation between HLB and the influence of emulsifier on functional properties. Our results are in accordance with those of Gontard et al. (16), whose investigation reported that the addition of sucroester of palmitic acid improved resistance of gluten films, whereas sucroester of stearic acid did not positively influence the mechanical properties of the film.

For GMS films, which showed similar WVP (Table 3), the formulation containing 10% L and 10% EM may be considered optimum because it gave the highest TS and E values. The 20%



**Figure 4.** ESEM cross-section micrographs of 20% cocoa butter films with 30% GMS (a), Tween 60 (b), and Tween 80 (c) and of 10% soybean oil films with 30% GMS (d).

**Table 4.** Variance (*F* Value) of Lipid Content (L) and Emulsifier Content (EM) on Tensile Strength (TS) and Elongation (*E*) of Edible Film Containing Cocoa Butter or Soybean Oil Related to Emulsifier Type (GMS, Tween 60, and Tween 80)

surfactant	source	<i>F</i> value <sup>a</sup>			
		soybean oil		cocoa butter	
		TS (MPa)	<i>E</i> (%)	TS (MPa)	<i>E</i> (%)
GMS	L	1.58	81.58**	34.59**	47.58**
	EM	0.17	6.06	2.94	18.12**
	interaction L–EM	0.03	2.30	0.15	8.9*
Tween 60	L	9.71*	0.63	12.01*	20.61**
	EM	48.92**	14.62*	16.63*	12.58*
	interaction L–EM	2.70	1.44	0.1	17.13*
Tween 80	L	116.37**	20.69**	369.22**	0.70
	EM	23.85**	7.05	1.02	3.73
	interaction L–EM	4.06	0.28	29.99**	6.34

<sup>a</sup>\*\*, significant at  $p \leq 0.01$ ; \*, significant at  $p \leq 0.05$ .

L and 10% EM formulation may be a good compromise of WVP and mechanical properties for Tween 60 samples. The lowest WVP of Tween 80 films was observed for the formulation containing 20% L and 30% EM (Table 3), but this formulation had low TS (10.71 MPa) and *E* (3.70%), probably because of lipid phase separation. However, the TS of Tween 80 film was close to that of the optimized GMS formulation, but *E* and WVP were lower. For Tween 80, good mechanical properties and WVP can be assured by adopting 10% L and 30% EM.

**Effect of Soybean Oil and Emulsifiers on Mechanical Properties.** The addition of emulsifiers to soybean oil films would be justified only if it improved mechanical properties, because it entails a slight rise in WVP (Figure 3).

Lipid and emulsifier contents had a significant effect on tensile strength for Tween 60 and Tween 80 films, but no

**Table 5.** Tensile Strength (TS) and Elongation (*E*) of Edible Film Containing Cocoa Butter Related to Lipid Content (L), Emulsifier Content (EM), and Emulsifier Type (GMS, Tween 60, and Tween 80)

	EM (%)	L (%)		mean
		10	20	
<b>TS<sup>a</sup> (MPa)</b>				
GMS	10	11.89 ± 0.73	9.06 ± 0.98	10.74
	30	10.94 ± 0.22	8.46 ± 0.33	9.70
	mean	11.42 a	8.76 b	
Tween 60	10	9.92 ± 1.10	8.56 ± 0.45	9.24 A
	30	8.29 ± 0.06	6.66 ± 0.30	7.48 B
	mean	9.11 a	7.61 b	
Tween 80	10	20.64 ± 1.17 Aa	8.62 ± 1.70 Ab	14.63
	30	18.21 ± 0.49 Aa	10.71 ± 0.81 Ab	14.46
	mean	19.42	9.66	
<b><i>E</i><sup>a</sup> (%)</b>				
GMS	10	28.63 ± 3.15 Aa	13.56 ± 1.04 Ab	21.09
	30	17.59 ± 2.12 Ba	11.62 ± 1.77 Aa	14.60
	mean	23.11	12.59	
Tween 60	10	28.13 ± 1.52 Aa	19.04 ± 1.73 Ab	23.58
	30	20.08 ± 0.10 Ba	19.66 ± 1.86 Aa	19.86
	mean	24.10	19.34	
Tween 80	10	4.27 ± 0.37	6.04 ± 1.37	5.16
	30	4.58 ± 0.41	3.70 ± 0.19	4.14
	mean	4.42	4.87	

<sup>a</sup> Means ± standard deviations. Means with the same capital letter in a column are not significantly different ( $p < 0.05$ ); means with the same small letter in a row are not significantly different ( $p < 0.05$ ).

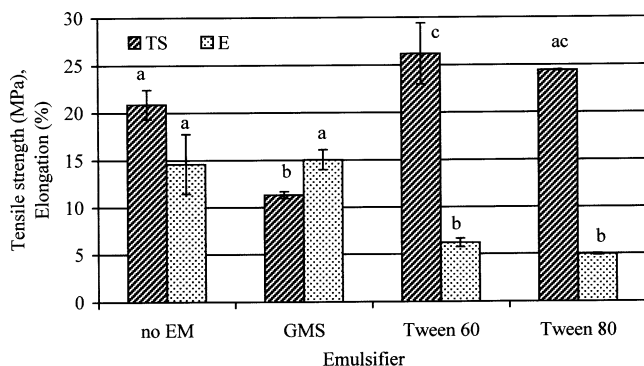
significant effect was observed for GMS samples (Table 4). Elongation was significantly influenced by L in GMS and Tween 80 films and by EM in Tween 60 films (Table 4).

For Tween 60–soybean oil formulations, an increase in L or EM lowered TS. An increase in EM caused *E* to fall (Table

**Table 6.** Tensile Strength (TS) and Elongation (E) of Edible Film Containing Soybean Oil Related to Lipid Content (L), Emulsifier Content (EM), and Emulsifier Type (GMS, Tween 60, and Tween 80)

	EM (%)	L (%)		mean
		10	20	
<b>TS<sup>a</sup> (MPa)</b>				
GMS	10	11.32 ± 0.36	10.42 ± 1.50	10.87
	30	11.13 ± 1.46	9.22 ± 0.5	10.18
	mean	11.22	9.82	
Tween 60	10	26.18 ± 3.20	19.93 ± 1.23	23.05 A
	30	14.84 ± 1.16	12.91 ± 0.81	13.87 B
	mean	20.51 a	16.42 b	
Tween 80	10	22.84 ± 1.15	15.74 ± 0.94	19.29 A
	30	24.43 ± 0.05	19.56 ± 0.49	21.99 B
	mean	20.50 a	17.65 b	
<b>E<sup>a</sup> (%)</b>				
GMS	10	15.05 ± 1.05	5.97 ± 0.29	10.51
	30	11.63 ± 1.92	5.14 ± 0.60	8.38
	mean	13.34 a	5.55 b	
Tween 60	10	6.22 ± 0.46	7.63 ± 0.94	6.92 A
	30	4.36 ± 0.49	4.07 ± 0.30	4.21 B
	mean	5.29	5.85	
Tween 80	10	5.54 ± 0.09	4.64 ± 0.25	5.09
	30	4.97 ± 0.16	4.26 ± 0.40	4.61
	mean	5.25 a	4.45 b	

<sup>a</sup> Means ± standard deviations. Means with the same capital letter in a column are not significantly different ( $p < 0.05$ ); means with the same small letter in a row are not significantly different ( $p < 0.05$ ).



**Figure 5.** Tensile strength (TS) and elongation (E) of 10% soybean oil films with no EM and with 10% GMS and Tween 60 and 30% Tween 80. Values are means, and standard deviations are represented by bars. Means with the same letters are not significantly different ( $p < 0.05$ ); statistical analyses are made independently for TS and E.

6). In the case of Tween 80, mechanical properties were reduced as L increased and high emulsifier content slightly enhanced tensile strength (Table 6). The increase in L was detrimental for E in GMS samples. As observed for cocoa butter films, there was no direct correlation between emulsifier HLB and the influence of EM on functional properties.

For GMS and Tween 60 films, the formulation containing 10% L and 10% EM may be considered optimum because it gave high TS and E values. In the case of Tween 80, the sample with the lowest WVP (10% L and 30% EM) also presented the best mechanical properties.

Figure 5 shows TS and E of the above optimized soybean oil-EM films and of the control (without EM) to assess if EM addition is beneficial for mechanical properties. GMS was the emulsifier that gave the lowest TS and the highest E. TS of soybean oil films without EM was lower than that of the same formulation containing Tween 60 and Tween 80, whereas E

values were lower in the presence of these emulsifiers. It seems that Tweens linked hydrophobic and hydrophilic film components or polysaccharide-polysaccharide chains more than GMS.

On the basis of these results, emulsifiers did not improve either WVP or mechanical properties of soybean oil films. Their use is therefore not advised.

**Conclusions.** Cocoa butter and soybean oil are mainly triglyceride-based lipids. Cocoa butter has a higher melting point than soybean oil, because it contains a greater proportion of saturated and monounsaturated esterified fatty acids. Only the addition of oil to the starch-MC-glycerol film-forming dispersion created an edible barrier that was more resistant to water vapor. Cocoa butter was ineffective, because it promoted the formation within the film of discontinuities, which were probably crystallization-related.

GMS, Tween 60, and Tween 80 used at optimal concentrations matched the WVP values of films containing the two lipids. Surfactants eliminated the imperfections (internal cavities) of the film with added cocoa butter, making it effective as a barrier to water vapor. Water vapor barrier properties of oil films containing EM were inferior to those of the same formulations without EM because surfactants increase the polarity of the lipid phase, which promotes water molecule sorption and water vapor mobility. For this reason, the WVP of edible films cannot be reduced simply by adding a hydrophobic component to the formulation. It is important to ascertain the effect of lipids on film microstructure.

From the results obtained, there appears to be no direct relationship between HLB and EM effects. Other factors, which require further study, intervene in the organization of the complex network of polysaccharides and lipids.

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