Effects of lipids on mechanical and moisture barrier properties of edible gellan film

L. Yang1, A.T. Paulson *

Department of Food Science and Technology, DalTech, Dalhousie University, Halifax, NS, Canada B3J 2X4

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Abstract

Beeswax or a 1:1 blend of stearic–palmitic acids (S–P) were incorporated into gellan films through emulsification to form gellan/lipid composite films. The films, containing 0–25% lipid (dry film basis) were examined for mechanical properties, water vapor permeability (WVP) and opacity. Addition of the lipids to gellan films significantly improved the WVP ($P < 0.05$), but lowered the mechanical properties and caused the films to become opaque. Beeswax was more effective than S–P acids in reducing the WVP, and films with beeswax showed better mechanical properties overall than those with S–P acids. The tensile properties of gellan films containing 14.3% beeswax were evaluated as a function of water activity ($a_w$) of the film. Increasing the $a_w$ decreased the tensile strength (TS) and elastic modulus but tensile elongation was not affected. The extent of the decreases in TS for the composite film was less pronounced than that for a similar film without lipids, suggesting that lipids help to alleviate moisture sensitivity of gellan films. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Water activity ($a_w$) is a critical factor affecting the sensory quality and shelf life of food products. During storage of foods, many deteriorative chemical and enzymatic reactions (lipid oxidation, Maillard browning and enzymatic browning) as well as microbial growth proceed at rates highly associated with water activity and water content of the foods (Labuza & Saltmarch, 1981; Lenovich, 1987; Leung, 1987). In addition, the textural properties of certain foods also largely depend on their water activity. Dry and crispy cereal foods may become soggy and lose their crispness upon water absorption, and soft-textured foods may become hard upon loss of water. Control of moisture exchange with the surrounding environment has been achieved in most instances through synthetic film packaging. However, with sufficient mechanical and barrier properties, biodegradable and/or edible films made from biological macromolecules, such as polysaccharides, proteins and lipids, could be used as replacements for synthetic films.

The functionalities and applications of edible films have been reviewed by Kester and Fennema (1986); Gennadios, Weller, McHugh and Krochta (1993) and Gontard and Guilbert (1994). In general, mechanical and barrier properties of biological films are highly associated with the polarity of film constituents (Debeaufort, Martin-Polo & Voilley, 1993; Fennema, Donhove & Kester, 1994). Most single hydrophilic films (polysaccharide or protein based films) have good mechanical properties and are excellent gas, aroma and lipid barriers but poor moisture barriers. On the other hand, most single hydrophobic (lipid-based) films have rather poor mechanical properties but high moisture resistance. Several investigators have attempted to improve the moisture barrier properties of hydrophilic films by incorporation of lipids through emulsion or lamination technology (e.g. Donhove & Fennema, 1992; Fennema et al., 1994; Gontard, Duche, Cuq & Guilbert, 1994; Greener & Fennema, 1989a,b; Haggermaier & Shaw, 1990; Handa, Gennadios, Hanna, Weller & Kuroda, 1999; Kamper & Fennema, 1984a,b; Kester

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& Fennema, 1989; Koelsch & Labuza, 1992; Shellhammer & Krochta, 1997). Among the lipid materials investigated, waxes and long-chain saturated fatty acids and fatty alcohols were most able to effectively improve the moisture barrier properties of hydrophilic films. However, it has also been reported that incorporation of certain lipid materials into hydrophilic films has an adverse effect on the mechanical and optical properties of the films.

Gellan, a polysaccharide secreted by the microorganism *Sphingomonas elodea* (formerly referred to as *Pseudomonas elodea*), forms an elastic gel in the presence of cations, and with an appropriate plasticizer the gel can be dried to form a film. In a previous study, biodegradable/edible gellan films with good clarity and mechanical properties were been developed, and their basic mechanical and moisture barrier properties were evaluated (Yang & Paulson, 2000).

The major objectives of this study were to develop gellan/lipid composite films through emulsification and to determine the effect of the lipids (beeswax and 1:1 blend of stearic–palmitic acids) on the moisture barrier, mechanical and optical properties of the films.

2. Materials and methods

2.1. Materials

Food grade deacylated gellan gum was donated by Nutrasweet Kelco, Monsanto Co., San Diego, CA. Glycerol, white beeswax, stearic and palmitic acids were purchased from Sigma Chemical Co., St. Louis, MO. Magnesium nitrate and CaSO4 desiccant (Drierite®, indicating, 8 mesh) were purchased from BHD Inc., Toronto, ON, Canada.

2.2. Preparation of gellan/lipid composite films

Gellan film-forming solutions (2%, w/v) were prepared by dissolving 3.0 g gellan gum in 150 ml distilled water with constant stirring, followed by heating to 95–100°C on a hot plate. 6.0 g glycerol (plasticizer) and an appropriate amount of lipid were added to the solution during heating and stirring. After the lipid melted, the hot solution was immediately emulsified with a high speed Polytron Homogenizer (Model PT10/35, Kinematica GmbH, CHE) for 3 min at 27,000 rpm. The emulsion (above 70°C) was then poured onto leveled and pre-warmed glass plates framed by four glass bars (1.55 mm thick) and spread evenly with a glass rod. The glass bars were removed after the gel set, and the plates were then dried in an oven at 40°C for 48 h. The finished intact films were peeled from the glass plates, and all film samples were conditioned in an environmental chamber at 22±1°C and 54% RH for 3 days prior to testing. The thickness of conditioned composite films ranged from 0.07 to 0.09 mm. All treatments were made in duplicate.

2.3. Tensile tests

Tensile strength (TS), percentage tensile elongation (TE) at break and elastic modulus (EM) of the film were determined at 21±1°C using an Instron Universal Testing Machine (Model 4502, Instron Corp., Canton, MA) according to ASTM standard method D882-91 (ASTM D882-91, 1991). Seven film specimens, 8×2.5 cm strips, were cut from each preconditioned (54% RH) film and mounted between the tensile grips. The initial grip separation and cross-head speed were set to 50 mm and 300 mm/min, respectively. A microcomputer was used to record the stress–strain curves. Elastic modulus is defined as the ratio of stress to strain in the initial linear part of the stress–strain curve, and is a fundamental measurement of film stiffness.

2.4. Puncture tests

Puncture strength (PS) and deformation (PD) of the film at rupture were determined using a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) as described by Gontard, Guilbert and Cuq (1992). Three specimens (4 cm diameter discs), were cut from each preconditioned film. Each specimen was mounted on top of a cup and a smooth edged cylindrical probe (0.8 cm i.d.) was moved perpendicularly on the film surface at a constant speed (1 mm/s) until the film broke. A force–deformation curve was recorded with a microcomputer for determining force and deformation at rupture.

2.5. Water vapor permeability

Water vapor permeability (WVP) of film was determined gravimetrically at 21±2°C using the ASTM standard method E96-93 (ASTM E96-93, 1993), and the WVP values were calculated using the WVP Correction Method described by Gennadios, Weller and Gooding (1994). Two specimens, 4 cm diameter discs, were cut from each film. Each specimen was sealed, with the smooth and shiny side facing up, by a rubber O-ring to the test cup (Plexiglas) containing a 10 ml saturated magnesium nitrate solution (54% RH) leaving a 17.95 mm air gap beneath the film, and placed in an air-tight plastic desiccator containing CaSO4 desiccant (0% RH). The cups were 4.50 cm (i.d.), 5.00 cm (o.d.) and 2.65 cm (depth) with an exposed film area of 15.90 cm². The water vapour transmission rate through the test films was determined from weight loss of the cups over time. The cups were weighed to the nearest 0.1 mg at 5 h intervals. The steady state of weight loss was reached after 5 h.
2.6. Film opacity

Opacity of the films was determined using a spectrophotometer (Model PU 8800 UV/VIS, Pye Unicam Ltd., Cambridge, UK) according to the procedure described by Gontard et al. (1994). Three film specimens (1×4 cm strips) were taken from each film sample and placed on the inner side of a transparent plastic cuvette. The absorption spectrum of the sample was obtained from 400 to 800 nm. Film opacity was defined as the area under the recorded curve which was obtained through an integration procedure, and was expressed as Absorbance units×wavelength product (Å×nm).

2.7. Film thickness

A micrometer (Model CD-6”BS, Mitutoyo Co., Japan) was used to measure the thickness of the conditioned films and the gap depth in the WVP test to 0.001 mm. Six random measurements for each film and gap depth were taken and averaged.

2.8. Scanning electron microscopy

Film samples were examined for surface characteristics using a Jeol 35C scanning electron microscope (SEM) (Jeol Ltd., Tokyo, Japan) operated at 10 kV. Film samples were affixed to aluminum stubs with doublesided cellophane tape and left at high vacuum overnight. The samples were then sputter-coated with a layer of gold prior to imaging.

2.9. Statistical analysis

Systat (SPSS Inc., Chicago, IL) was used for all statistical analyses. Analysis of variance and Bonferroni multiple comparisons were performed to detect significant differences in film properties at the 5% confidence level.

3. Results and discussion

3.1. Film formation

The maximum amount of the lipids that could be added to gellan films appeared to be ~25% of the film dry weight [lipid/(lipid + gellan + glycerol)]. Films containing more than this amount had an uneven lipid distribution which most likely arose from the limited dispersion capability of the lipids and from the poor stability of the emulsion systems at higher lipid concentrations.

Small losses of lipids upon removal of the films from the glass plate were observed in this study, but were not quantified. However, other problems often encountered in formation of composite films, such as distortion, cracking and pinhole formation during oven drying (e.g. Kamper & Fennema, 1984a,b; Vojdani & Torres, 1990), were not observed for gellan/lipid composite films.

The appearance of the two composite films with and without vacuum application during film formation was compared in preliminary experiments. It was noticed that deaeration of the emulsified film-forming solutions deteriorated, to some extent, stability of the emulsion systems, leading to an uneven distribution of the lipids in the film matrix, hence an inconsistent appearance. Therefore, deaeration should be avoided during formation of gellan/lipid “emulsion” films. Nonetheless, without deaeration, small pores could be present within the film, which may result in an elevated WVP (McHugh, Aujard & Krochta, 1994).

3.2. Water vapor permeability (WVP)

WVP of the gellan/lipid composite films at various lipid concentrations was evaluated at 0/54% RH. As shown in Fig. 1, addition of the two lipids to gellan films caused an essentially linear reduction in film WVP. WVP of the films containing 20% S–P acids or 20% beeswax was decreased by 20 and 53%, respectively, as compared with that of gellan film without lipid. At the same lipid level, WVP of films with beeswax was significantly (P<0.05) lower than that of films with S–P acids.

In general, WVP of composite films comprised of biopolymers and lipids strongly depend on the type, structure and quantity of the lipids. For films containing fatty acids or fatty alcohols, the WVP decreased with increased chain-length and degree of saturation of the lipids (Hagenmaier & Shaw, 1990; Kamper & Fennema, 1984a,b; Koelsch & Labuza, 1992; McHugh & Krochta,

![Fig. 1. Water vapor permeability (WVP) of gellan/lipid composite films as a function of lipid concentration (error bars are standard error of the mean of two measurements from two separate films).](image-url)
1994a). Kamper and Fennema (1984a,b) examined the effect of fatty acids on the WVP of hydroxypropyl methylcellulose (HPMC) film, and indicated that stearic acid and a 1:1 blend of stearic–palmitic acids were the most effective fatty acids in reducing WVP of the films. They observed no significant difference in barrier efficiency between the two composite films at the same lipid concentration. Therefore, S–P acids was recommended for use in hydrophilic films since these composite films are easier to prepare in a laboratory situation (low melting point) and have a more consistent appearance than other lipids.

Beeswax was also reported to be an excellent moisture barrier for wheat gluten films, whey protein films, and cellulose-based films (Avena-Bustillos & Krochta, 1993; Fennema et al., 1994; Gontard et al., 1994; McHugh & Krochta, 1994b; Shellhammer & Krochta, 1997). In this study, a 1:1 blend of stearic–palmitic acids as well as beeswax were chosen to evaluate the effect of lipids on the properties of gellan/lipid composite films because of their reported high moisture barrier efficiency, edibility and ease in preparation as well as low cost. The results revealed that both beeswax and S–P acids, at appropriate concentrations, can effectively reduce the WVP of gellan films, but beeswax was more effective. This agreed with the study of Avena-Bustillos and Krochta (1993) for caseinate–lipid composite films. Similar results have also been reported by Kester and Fennema (1989) and Fennema et al. (1994) who compared the permeance of various lipid films to moisture, and found that beeswax film had a significantly ($P < 0.05$) lower permeance to moisture than stearic acid film at 0/100% RH. According to their hypothesis, the difference in barrier efficiency between S–P acids and beeswax could be attributed to their different polarity. Although fatty acids are considered to be hydrophobic substances, their molecules contain highly polar carboxyl groups that may interact with water molecules and consequently facilitate moisture transfer through the film. Beeswax is a blend of lipid molecules with desirable non-polar properties. Esters of long-chain fatty alcohols and acids are the major components, and long-chain hydrocarbons, free fatty acids and fatty alcohols are the minor components. Even though polar groups (carbonyl groups of esters, carboxyl groups of fatty acids and hydroxyl groups of fatty alcohols) are also present in beeswax, they are essentially buried within the matrix of long hydrocarbon chains, and thereby contribute little polarity (Fennema et al., 1994).

It has previously been reported that film orientation in the permeability tester has a significant effect on WVP of composite films resulting from partial emulsion separation (lipid creaming) during film formation. Emulsion film oriented with the lipid-enriched side facing toward the higher RH environment exhibited a lower WVP than film oriented with the lipid-enriched side facing toward the lower RH environment (Avena-Bustillos & Krochta, 1993; McHugh & Krochta, 1994a). In this study, lipid-enriched sides of the composite films were faced down to the high RH during the WVP measurements.

### 3.3. Mechanical properties

Adequate mechanical strength and extensibility are generally required for a packaging film to withstand external stress and maintain its integrity as well as barrier properties during applications in packaging. Research on lipid/hydrocolloid composite films has focused mainly on their moisture barrier properties. The influence of lipid incorporation on the mechanical properties of hydrocolloid films has not been extensively investigated.

The TS of the composite films decreased sharply as concentration of the lipids increased (Fig. 2). For example, TS of the films with 14.3% S–P or beeswax was decreased by 34% as compared with that of the film without lipid. No significant difference ($P < 0.05$) in TS occurred between the two composite films at lipid concentrations below 14.3%. However, at lipid concentrations above 14.3%, the films with beeswax had slightly higher TS than the films with S–P acids. Tensile elongation (TE) of the composite films with S–P acids decreased as the concentration of S–P increased, whereas TE of the films with beeswax changed little as the concentration of beeswax increased (Fig. 3). Addition of the two lipids also resulted in marked decreases in both puncture strength (PS) and puncture deformation (PD) of the films as shown in Figs. 4 and 5. As with TS, at lipid concentrations below 14.3%, no significant differences in PS and PD were observed between the two

![Fig. 2. Tensile strength of gellan/lipid composite films as a function of lipid concentration (error bars are standard error of the mean of seven measurements from two separate films).](image-url)
films ($P > 0.05$), whereas at lipid concentrations above 14.3%, the films with beeswax appeared to have no further decreases in PS and PD, but the films with S–P acids showed further decreases in both PS and PD. With respect to both tensile and puncture properties, the composite films with beeswax seemed to exhibit better mechanical properties than the films with S–P.

These results agreed with the study conducted by Gontard et al. (1994) in which they found that, for whey protein/lipid composite films, the influence of lipids depended on lipid characteristics and interactions between the lipid and the protein structural matrix, and both PS and PD of the composite films decreased rapidly as beeswax concentration increased. However, Park, Weller, Vergano and Testin (1993) examined the tensile properties of fatty acid-laminated MC films, and reported that lamination of fatty acids on the surface of MC films reduced their TS, but increased the TE. The trends of the tensile properties as a function of the concentration of fatty acids were dependent on the characteristics of the fatty acids.

The negative effects of the lipids on mechanical properties of the gellan/lipid composite films may have resulted from the partial replacement of gellan polymers by the lipids in the film matrix. The interactions between non-polar lipid molecules and between polar polymer and non-polar lipid molecules are believed to be much lower than those between the polar polymer molecules. The differences in mechanical properties between the two films could be related to their different polarity. Beeswax is a highly hydrophobic substance which consists of 71% wax esters, 1–1.25% free fatty alcohols, 13.5–14.5% free fatty acids, 10.4–13.6% hydrocarbons, and 1–2% moisture and unknown matter (Tulloch & Hoffman, 1972). Relatively polar fatty acids are less hydrophobic than beeswax. The hydrophobic characteristic of beeswax may have resulted in a relatively lower water concentration in the composite film, particularly at high lipid concentration, than that of more hydrophilic S–P films when exposed to the same equilibrated RH. Therefore, a lower water concentration in the composite films with beeswax may be responsible for their lower WVP and higher mechanical strength. Furthermore, commercially available deacylated gellan gum contains small amounts of mono- or divalent cations for inducing gel formation (Chalupa, Colegrove, Sanderson & Valli, 1994). A large number of carboxyl groups on the molecules of S–P acids may compete with those on the gellan molecules for the cations in the film-forming solution, reducing the interactions between the gellan polymer...
chains and hence weakening mechanical strength of the film. In consideration of both mechanical and moisture barrier properties, 14.3% (w/v) beeswax in film-forming solution is recommended for production of gellan/lipid composite films.

3.4. Opacity

Transparency (low opacity) of films is of importance, in some instances, when used as package materials. Addition of lipid generally caused the films to become whitish. Gellan film without lipids was rather transparent. However, incorporation of S–P acids or beeswax into the films resulted in a sharp increase in the film opacity, and the opacity increased as concentration of the lipids increased (Fig. 6). The increase in film opacity probably arose from light scattering from lipid droplets which were dispersed in the emulsion and continuously distributed throughout the polymer network after the film formed. It was also observed that the film with beeswax was more opaque than the film with S–P acids at the same lipid concentration. This was most likely due to differences in optical properties of the two types of lipids.

3.5. Surface characteristics

To help elucidate the relationship between surface characteristics and moisture barrier properties of the films, SEM was used to examine the surface of the lipid-enriched side of composite films with 14.3% lipids as well as a gellan film with no lipids. The micrographs (Fig. 7) show increased surface irregularity with the addition of lipids. However, beeswax (Fig. 7b) appeared to be well incorporated and embedded in the gellan matrix resulting in a relatively smooth and continuous surface, whereas the film with S–P acids (Fig. 7c) had a highly irregular surface which may have resulted from greater creaming of S–P acids in the film-forming solution prior to setting of the gellan gel. It is unlikely that the irregular features were due to entrapped air bubbles since each type of film was made by the same procedure. The differences in surface structure may have contributed to some extent to the differences in WVP of the films.
two films. The more highly irregular S–P acids film may have had a relatively greater effective surface area and shorter effective film thickness for water vapour transmission than the film with beeswax, resulting in higher WVP.

3.6. Effect of water activity on the tensile properties of gellan/beeswax composite film

Tensile properties of the gellan composite films with 14.3% beeswax over a wide range of water activity (aw) were examined. TE of the films appeared to be relatively independent of aw over the aw range tested. Increasing aw caused continuous decreases in TS and EM (Fig. 8), which was similar to that found for gellan film without lipids (Yang & Paulson, 2000). However, the extent of the decreases in TS and EM for the composite films was less pronounced than for gellan film without lipids. For example, TS of the film without lipids decreased by 45% as the aw increased from 0.11 to 0.54, whereas TS of the gellan/beeswax composite film decreased by 31%. Thus, addition of the beeswax helped alleviate the sensitivity of gellan films to moisture. However, it should also be stressed that the composite films remained intact and functional up to aw ~0.9. Above aw 0.90, they will extensively absorb water, become softer and may lose their integrity, which would consequently result in markedly elevated WVP and reduced mechanical strength. As previously described, beeswax is a nonpolar hydrophobic substance, but a small number of polar groups, such as the ester groups of the wax, hydroxyl groups of the fatty alcohols and carboxyl groups of free fatty acids, are hydrophilic. Although the hydroxyl and carboxyl groups are buried within the aliphatic chains, they may, at very high RH, still be able to absorb a small number of water molecules and hydrate sufficiently to influence the functional properties of the film. This hydration would be expected to cause some swelling of the film matrix and changes in film functionality.

4. Conclusions

Gellan/lipid composite films with improved WVP relative to gellan films without lipids can be made through emulsion technology. However, the film mechanical properties are lowered and the films become more opaque. Beeswax was more effective than S–P acids in reducing the WVP, and films with beeswax showed better mechanical properties overall than those with S–P acids. Differences in surface characteristics of the films suggest that surface microstructure may be partially responsible for differences in WVP of gellan/lipid composite films. Although increased water activity weakened gellan/beeswax composite films, the effect was less than with gellan films without lipids suggesting that lipid incorporation can help alleviate moisture sensitivity of gellan films.

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References
