Influence of the Addition of Lauric Acid to Films Made from Gelatin, Triacetin and a Blend of Stearic and Palmitic Acids

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Summary: The objective of this research was to verify the influence of adding increasing amounts of lauric acid on the functional properties of homogenized films made from gelatin, triacetin and a blend of palmitic and stearic acids. The films were characterised with respect to their visual aspect, water vapour permeability (WVP), water solubility, mechanical properties (tensile strength and percent elongation), oxygen permeability (O_2P), opacity (OP) and melting and glass transitions temperatures. The films produced were malleable and macroscopically homogeneous. The addition of 1% of lauric acid to the film of gelatin, triacetin and blend of palmitic and stearic acids (5.84 ± 0.31 gmm $\cdot$ m^{-2} dkPa) caused a slight decrease in WVP. The additions of 2.5% (5.70 ± 0.76 gmm $\cdot$ m^{-2} dkPa), 5% (5.38 ± 0.64 gmm $\cdot$ m^{-2} dkPa) and 10% (4.50 ± 0.55 gmm $\cdot$ m^{-2} dkPa) of lauric acid were sufficient to make a significant difference in the WVP at the higher levels used. As compared to the gelatin and triacetin film, the addition of lauric acid at all the concentrations studied resulted in a slight increase in the film solubility. The addition of hydrophobic substances to gelatin/triacetin films (15.26 ± 0.28 cm^3 $\cdot$ μm $\cdot$ m^{-2} dkPa) favoured an increase in O_2P permeability, this effect being greater in the films made from gelatin, triacetin, blend of palmitic and stearic acids and 10% lauric acid (24.48 ± 0.07 cm^3 $\cdot$ μm $\cdot$ m^{-2} dkPa). The increasing addition of lauric acid significantly reduced the tensile strength and increased elongation of the films composed of gelatin, triacetin and blend that being more evident at the concentrations of 5% (67.58 ± 1.23 MPa and 11.45 ± 0.57%) and 10% (63.50 ± 1.56 MPa and 12.90 ± 0.57%). The addition of 1% (OP, 27%) and 10% (OP, 28%) of lauric acid induced no visible effect on the opacity of the films. The thermogrammes showed three transitions for the gelatin/triacetin/stearic-palmitic blend/1% lauric acid films (-57.42 °C, 23.74 °C and 44.11 °C) and two for the gelatin/triacetin/stearic-palmitic acids blend/10% lauric acid films (-56.22 °C and 17.35 °C). As observed by DSC, the addition of fatty acids resulted in the appearance of more than one melting peak for all films in relation to the gelatin and triacetin film.

Keywords: barrier; biodegradable films; blends; fatty acids; glass transition

Introduction

Films can be classified as edible and/or biodegradable depending on the constituents used to produce them and the amount of each substance employed.[17] Their preparation involves the use of various components, each with a specific purpose. Such formulations consist of at least one film forming agent (macromolecule), a solvent (water, ethanol, water/ethanol amongst others), a plasticiser (glycerol, sorbitol etc.) and in some case a pH-adjusting agent (acetic acid, NH_4OH etc.). The use of this technology dates from the

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XII and XIII centuries for the coating of citric fruits.[11] These films are used in a variety of applications, although the available technical information has not yet reached an optimal state, thus requiring further research to develop specific films and coverings for each kind of food.[8] Each group of materials used in the formulation of such films presents advantages and disadvantages, it being desirable to use a combination to produce new films.[4] A combination of proteins or polysaccharides with lipids appears to show advantages over films produced with only one of these components, what improves their functional properties.[12] Proteins and polysaccharides form a continuous, cohesive network, giving adequate mechanical properties, but they are not the best barriers against water vapour due to their hydrophilic nature, limiting their use in products requiring a moisture barrier. On the other hand, lipids form films with good water vapour barrier properties, due to their hydrophobic nature, but they are very fragile.[6] The objective of this research was to verify the influence of adding increasing amounts of lauric acid on the functional properties of homogenised films made from gelatin, triacetin and a blend of palmitic and stearic acids.

Materials and Methods

Materials

The protein source used was bovine hide type A gelatin (bloom = 244; particle size <6 mesh; protein = 89.0%; moisture content = 9.8%; viscosity 35 mPs) donated by Leiner Davis Gelatin Brazil (São Paulo, SP, Brazil). Triacetin (Lot number CAS 102.76.1) donated by Rhodia, (Paulinia, SP, Brazil). Palmitic, stearic and lauric acids, magnesium chloride ($Mg(NO_3)_2$-$6H_2O$), sodium chloride (NaCl) and sodium sulphate (Na_2SO_4) were donated from Vetec (São Paulo, SP, Brazil).

Preparation of Composite Films with Fatty Acids

The film-forming solution was prepared by hydrating 10 g of gelatin (GEL) in 100 mL of distilled water, for 1 h at room temperature. After this, the solution was warmed at 90 °C per 10 min in water bath. Triacetin (15% w/w dry gelatin) plus stearic-palmitic fatty acids blend (10% each one; w/w dry gelatin) and lauric acid (1, 2.5, 5 and 10% w/w dry gelatin) were heated until it became completely homogeneous. The solutions were mixed and stirred using a magnetic stirrer, poured and spread evenly over 15 cm diameter plexiglass plates, and then dried at room temperature for 24 h. The quantity of solution poured onto the surface was calculated to obtain a constant thickness of the dried film. All films used for experiments were equilibrated at 52% relative humidity (RH) at 25 °C, and left standing 48 h before being tested.

Methods

Film Characterization

Visual Aspect: Visual and tactile analyses were carried out with each type of film, with the aim of using only homogenous films with respect to the absence of insoluble particles, uniform color, without rupture or brittle zones and easy to remove from the Plexiglass support. Films not presenting these characteristics were discarded.

Thickness: Film thickness was determined using a Mytutoyo Corp. digital micrometer (Tokyo, Japan) taking the average of 10 random measurements.

Water Vapor Permeability: The water vapor permeability (WVP) of the films was determined gravimetrically[1] at 25 ± 1 °C. The insides of the cells were filled with silica gel. The cells were covered with the films and sealed with paraffin. The cells were then placed in desiccators containing a saturated NaCl solution (75 ± 2% RH) and weighed (± 0.0001 g) daily to constant weight. WVP was measured in triplicate.

Soluble Matter: Water-soluble matter (WSM) was determined in triplicate.[10] Samples

(2.0 cm in diameter) were immersed in 50 mL distilled water and the system slowly stirred mechanically at $25 \pm 1\,^{\circ}C$ for 24 h. The initial mass was determined from the sample moisture content. After this period, the samples were removed from the solution and dried in a forced air oven (105 °C, 24 h), and the difference in weight used to calculate the water soluble matter as a percentage of initial weight.

Mechanical Properties: The tensile strength (TS) and elongation (E) of the films (100×25 mm), as the average of five determinations, were determined using a model TA-XT2 texturometer, TA Instruments (Newcastle, USA).[2] The initial distance of separation and velocity were adjusted to 50 mm and 1 $mm \cdot s^{-1}$ respectively. Before analysis, the samples were conditioned for 72 h at $50 \pm 2\%$ RH, $25 \pm 1\,^{\circ}C$.

Oxygen Permeability: Oxygen permeability (OP) was determined in duplicate at $25 \pm 1\,^{\circ}C$.[3] The oxygen transmission rate was determined in an OX-TRAN 2/20, Mocon Inc. (Minneapolis, Minn. USA). The samples were equilibrated at $50 \pm 2\%$ RH for a period of 72 h before analysis. The gas flow rate was fixed (10 $mL \cdot min^{-1}$) and the difference in pressure across the film (diameter of 2.5 cm) corresponded to the atmospheric pressure (101.3 kPa). An aluminum mask was used to facilitate film fixation. With this equipment it is not possible to control the relative humidity during the analysis.

Opacity: Film opacity was determined using a HUNTERLAB (Colorquest II, Reston, VA, USA). The determinations were made in triplicate after calibration using standard black and white backgrounds, where Opacity (OP) $= (O_{pb}/O_{pw}) \times 100$, OP being the % opacity of the film, O_{pb} = opacity of the film against a black background and O_{pw} the opacity of the film against a white background.

Dynamic Mechanical Calorimetry (DMTA): The glass transition temperatures were determined using a DMA 2980, TA Instruments (New Castle, DE, USA), equipped with a cryogenic system using liquid nitrogen. During the analysis a continuous flow of nitrogen through the oven provided an inert atmosphere. The sample size was 3.5 cm × 0.8 cm. Each sample was analyzed in duplicate. Tension and heating were applied to the sample simultaneously. Measurements were taken at a frequency of 1 Hz, amplitude of 20 μm and strength of 1 N in the temperature range from −100 to +150 °C with a heating rate of 2 $^{\circ}C \cdot min^{-1}$. The measurements of the storage modulus (E′), loss modulus (E″) and angle of loss (tan δ) were registered and plotted against the temperature for the analysis of the thermal transitions. The transition temperature was determined at the point of inflection of the curve of angle of loss (tan δ) as a function of temperature.[7]

Differential Scanning Calorimetry (DSC): The melting point/transition temperature (TM) and the respective enthalpy (ΔH) were determined by differential scanning calorimetry, using a DSC 2010, TA Instruments (New Castle, DE, USA) with a liquid nitrogen unit. Duplicate samples (10.0 to 15.0 ± 0.1 mg) were conditioned at $50 \pm 2\%$ RH, $25 \pm 1\,^{\circ}C$ for 7 d and the capsules were subsequently sealed hermetically. The samples were heated at a rate of 10 $^{\circ}C \cdot min^{-1}$ between 0 and 140 °C under an inert atmosphere (100 $mL \cdot min^{-1}$ N_2). Indium was used to calibrate the equipment. The maximum peak temperature of the endotherm was taken as the melting point/transition temperature.

Statistical Analysis

Statistical analyses were carried out using Statistica 5.5 (Statsoft, Tulsa, OK, USA) and the differences between the means determined by the Tukey multiple test ($p < 0.05$).

Results and Discussion

The films produced were malleable and visibly homogeneous. The addition of lauric

Table 1.
Water vapor permeability and water solubility of the films.

Films[a)]	Thickness	Water vapor permeability[b)]	Solubility[b)]
	mm	$gmm \cdot m^{-2}$ dKPa	%
Gel/Ac/Tri 15%	0.069 ± 0.012	5.84 ± 0.31^{a}	34.59 ± 0.77^{c}
Gel/Ac/Tri/La 1%	0.070 ± 0.004	5.80 ± 0.16^{a}	34.88 ± 0.11^{bc}
Gel/Ac/Tri/La 2.5%	0.071 ± 0.004	5.70 ± 0.76^{ab}	34.92 ± 0.73^{bc}
Gel/Ac/Tri/La 5%	0.071 ± 0.007	5.38 ± 0.64^{ab}	35.48 ± 0.45^{ab}
Gel/Ac/Tri/La 10%	0.070 ± 0.005	4.50 ± 0.55^{b}	36.74 ± 0.19^{a}

a) Gel: gelatin, Tri: triacetin, Ac: acids (1:1 stearic and palmitic acid), La: lauric acid.
b) Mean and standard deviation of the replicates. Note: different letters represent significant difference ($p < 0{,}05$) according to the TUKEY test.

acid to the film of gelatin, triacetin and blend of palmitic/stearic acids caused a decrease in WVP (Table 1). The additions of 2.5, 5 and 10% of lauric acid were sufficient to produce a significant difference in the WVP at the higher levels used. At a concentration of 10%, the reduction was more accentuated, indicating an increase in the film hydrophobicity, due to the addition of the fatty acid.[10] In the film forming solution, the proteins are partially immobilised at the interface with the emulsified lipid particles. Consequently, the polymeric chains became less mobile, reducing the diffusibility of water via the protein interface and leading to a decrease in WVP.[13]

The addition of 1 and 2.5% of lauric acid to the gelatin/triacetin/blend film did not produce statistically significant variations in the film solubility; an effect observed with the addition of 5 and 10% of lauric acid, although the variation range was not relevant (35.48 to 36.74%) (Table 1). The increase in water solubility of protein films on addition of lipid material is dependent on the amount added, and can partially destabilise the structure of the protein matrix by reducing the intermolecular forces between the protein chains.[10]

The increasing addition of lauric acid significantly reduced tensile strength and increased elongation of the gelatin/triacetin/blend films (Table 2), this being more evident at the concentrations of 5 and 10%. That indicates that the protein phase has greater tensile strength then the lipid phase. An increase in concentration of the lipid phase causes a reduction in the tensile strength of the protein matrix. An increase in lipid concentration can lead to increased film elongation, due to its plasticising effect.[16] The same effect was observed when 'brazilian elemi' was used to produce gelatin films.[5]

The addition of lauric acid (1%) to gelatin/triacetin/blend films favored an increase in O_2P (Table 2), this effect being greater at 10% lauric acid. A similar effect

Table 2.
Mechanical properties and oxygen permeability of the films.

Films[a)]	Tensile strength[b)]	Elongation[b)]	Oxygen permeability[b)]
	MPa	%	$cm^3\mu m \cdot m^{-2}$ dkPa
Gel/Ac/Tri 15%	91.41 ± 2.02^{a}	8.92 ± 0.66^{b}	21.92 ± 0.87^{b}
Gel/Ac/Tri/La 1%	85.17 ± 1.47^{b}	9.25 ± 1.36^{b}	23.13 ± 0.63^{ab}
Gel/Ac/Tri/La 2.5%	72.07 ± 1.57^{c}	10.57 ± 0.71^{ab}	–
Gel/Ac/Tri/La 5%	67.58 ± 1.23^{d}	11.45 ± 0.81^{ab}	–
Gel/Ac/Tri/La 10%	63.50 ± 1.56^{d}	12.90 ± 0.57^{a}	24.48 ± 0.07^{a}

a) Gel: gelatin, Tri: triacetin, Ac: acids (1:1 stearic and palmitic acid), La: lauric acid.
b) Mean and standard deviation of the replicates. Note: different letters represent significant differences ($p < 0{,}05$) according to the TUKEY test.

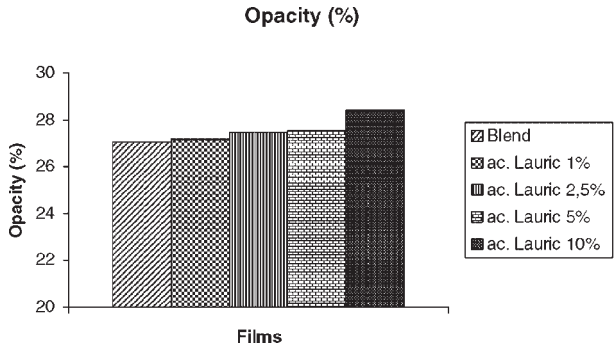

Figure 1.
Opacity of the films.

was observed, when working with films of gelatin and fatty acids.[9]

The addition of 1% (27% OP) and 10% (28% OP) of lauric acid increased the opacity of the gelatin/triacetin/blend film (25% OP) (Figure 1). Higher values of opacity were associated with the increasing amount of hydrophobic compounds.[15]

The glass transition temperatures (T_gs) of the films were determined by dynamic mechanical analysis (Table 3). The thermogrammes showed three transitions for the gelatin/triacetin/blend/1% lauric acid film [Figure 2(a)] and two for the gelatine/triacetin/blend/10% lauric acid films [Figure 2(b)]. The negative transition temperatures may be related to the transition of the plasticiser-rich fraction, indicating phase separation between the film forming agent (gelatin and fatty acids) and the plasticiser. The second glass transition temperature (T_g2) observed was related to the transition temperature of the protein-rich phase. T_{g3} is probably related to the transition of the fatty acids, due to the lack of physical accommodation of the acids in the filmogenic matrix. A widening of the transition peak was also observed on increasing the lauric acid concentration from 1 to 10%. The same phase separation was observed with simple gelatin plus triacetin films, a case where two glass transition temperatures were observed: one related to the transition of the plasticiser-rich fraction (−61 °C) and the other to the protein-rich fraction (28.45 °C).[5] As observed by DSC, the addition of fatty acids resulted in the appearance of more than one melting peak (Table 3) for all the films. These results suggest that the hydrophobic substances used were not completely incorporated into the gelatine matrix, a similar effect being observed for linear fatty acids with chain longer than ten carbons using gluten film.[14] The addition of 1 and 10% of lauric acid resulted in a decrease in the melting temperature peak of the fatty acids

Table 3.
Temperatures of glass transitions (T_g), temperature of melting (T_m) and enthalpy of melting.

Films[a)]	T_g			T_m			
	°C			°C			
	T_{g1}	T_{g2}	T_{g3}	T_{m1}	ΔH_{m1}	T_{m2}	ΔH_{m2}
					$J \cdot g^{-1}$		$J \cdot g^{-1}$
Gel/Ac/Tri	−56.36	27.48	–	58.10	22.98	91.10	5.71
Gel/Ac/Tri/La 1%	−57.42	23.74	44.11	56.38	22.46	91.81	4.57
Gel/Ac/Tri/La 10%	−56.22	17.35	–	43.11	25.26	89.78	5.51

[a)] Gel: gelatin, Tri: triacetin, Ac: acids (1:1 stearic and palmitic acids), La: lauric acid.

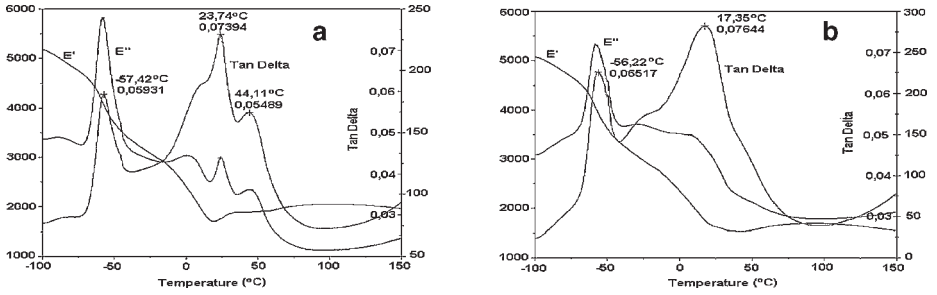

Figure 2.
Dynamic mechanical calorimetry (DMTA) of the films: (a) gelatin/triacetin/blend /lauric acid 1%, (b) gelatin/triacetin/blend/lauric acid 10%.

compared with the gelatin/triacetin/blend film (Table 3). This effect was more significant at the high concentration of lauric acid.

Conclusion

Addition of increasing amount of lauric acid to films composed by gelatin, triacetin and blend of palmitic/stearic acids has produced films malleable and apparently homogeneous. The addition of lauric acid to the films led to a decrease in the WVP and tensile strength, but to an increase in other parameters as O_2P, elongation, solubility in water and opacity.

As a general tendency, the addition of hydrophobic materials to hydrocolloids leads to a decrease of the barrier to water vapor as well as the mechanical properties, like the tensile strength. By the other hand, there is an increase in the opacity and the elongation effects. Particularly, all these effects depend on the polarity and molecular volume of the lipophilic material added, that determine the characteristics of the resulting matrix.

The homogenisation of the matrix containing hydrophobic compounds is an essential step to optimise the film functional properties. The present study showed that the addition of different concentrations of lauric acid to the film made by gelatin/triacetin/stearic-palmitic acid blend did not result in its homogenous incorporation to the filmogenic matrix. This critical step has to be improved.

Precedent studies using the same basic film matrix and the vegetal resinous hydrophobic exudates "elemi do Brasil" as the lipophilic materials have already evidenced this difficulty.[5] The lower polarity and higher molecular volume of the elemi constituents did not produced significant variations in the films, with regard to the water solubility, opacity and elongation, when compared to the addition of lauric acid. Films containing elemi presented a higher WVP barrier and a higher tensile strength; effects that can be attributed to their triterpenic composition. Nevertheless, either the addition of lauric acid or elemi to gelatin/triacenti/stearic-palmitic blend resulted in a non-homogeneous matrix, indicating the necessity of complementary studies.

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