Effect of saturated and unsaturated fatty acids on structural and optical properties of corn starch-glycerol based films

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ABSTRACT
Starch is one of the cheapest raw materials which can be used in edible film formation. Due to the fact that it is a natural constituent, its incorporation as a food coating does not involve any health risks, although as a barrier material it has the disadvantage of having poor water vapour barrier properties. This could be improved if different non-polar components are included in the starch matrix. In this sense, edible films were formulated using corn starch and different fatty acids and the impact of these lipids on the optical properties of the films (transparency and gloss) was studied. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) were also used in order to analyse the film microstructure and to gain a better understanding of the films’ optical parameters. Lipid containing films were the least transparent due to the presence of discontinuities in the films’ matrices (lipid aggregates). In this sense, it is remarkable that films containing oleic acid were the least transparent. SEM micrograph allows us to confirm the greater structural heterogeneity of these films, coinciding with the opacity development. Gloss of films (measured at different incidence angles) was also affected by lipid addition and type of fatty acid. Films containing stearic acid showed the highest gloss values, which agrees with that observed in the AFM images where these films also showed the smoothest surface. Films with palmitic acid showed a smoother surface than either the control films (without lipid) or the films containing oleic acid.

Keywords: Starch; fatty acids; transparency; gloss.

1. INTRODUCTION
Nowadays, consumer trends are changing continuously and terms such as sustainability or environmentally-friendly materials are becoming more widely appreciated. Traditional packaging materials are considered as waste when their main function has concluded. These large amounts of synthetic polymers need to be processed and recycled, which needs a significant investment on the part of every country. Thus, natural polymers constitute a “green” alternative to petroleum-based plastics due to their biodegradability and compatibility with food products. In this sense, proteins and polysaccharides have been used to obtain edible films and coatings which show adequate functional properties, which lead to a lengthening of the foodstuffs’ shelf-life. Starch is one of the polymers that is most widely used to obtain edible films or packagings. As other polysaccharides, starch produces films with adequate gas barrier properties. However, due to its hydrophilic nature, it acts as a poor barrier against water vapour transfer. To overcome this disadvantage, lipid materials are added in order to increase film hydrophobicity. Waxes and fatty acids can reduce water vapor permeability of films satisfactorily. However, these lipids can negatively modify other relevant properties of the films due to its phase separation during film drying. It has been observed that fatty acids can form a layered structure in sodium caseinate [1] and hydroxypropyl-methylcellulose [2] based films provoking a significant decrease in transparency and gloss. Taking these aspects into account, the influence of both saturated and unsaturated fatty acids on the optical properties of corn starch based films were analysed in this work.

2. MATERIALS & METHODS
Materials
Corn starch was obtained from Roquette (Roquette Laisa España, Benifaió, Spain). Glycerol and fatty acids, palmitic (PA, C<sub>16:0</sub>), stearic (SA, C<sub>18:0</sub>) and oleic acid (OA, C<sub>18:1</sub>), with a minimum purity of 96%, were purchased from Panreac Química, S.A. (Castellar Del Vallés, Barcelona, Spain).
Methods

Four different formulations, based on starch, glycerol and fatty acids, were prepared. Corn starch was dispersed in water in order to obtain 2% (w/w) polysaccharide suspensions. These dispersions were maintained, under stirring, at 95 ºC for 30 minutes until the starch gelatinization was completed. Subsequently, after tempering dispersions, the glycerol was incorporated in a starch:glycerol ratio of 1:0.25. Furthermore, evaporated water content was corrected. Afterwards, the dispersions were homogenised (13,500 rpm for 1 min and at 20,500 rpm for 5 min) at 95 ºC, under vacuum using a rotor-stator homogenizer (Ultraturrax T25, Janke and Kunkel, Germany). In the case of emulsions containing fatty acids (1:0.15 starch:fatty acid ratio), these were incorporated prior to the emulsifying step. After the homogenization step, films were prepared by weighing the amount of film-forming emulsion containing 1.5 g of total solids. This mass of emulsion was spread evenly over a Teflon casting plate (15 cm diameter) resting on a level surface, and films were formed by drying for approximately 48 hours at 45% RH and 20 ºC. Dried films could be peeled intact from the casting surface.

The transparency of films was measured through the surface reflectance spectra (400 – 700 nm), on white and black backgrounds, using a spectrocolorimeter MINOLTA, model CM-3600d (Minolta Co., Tokyo, Japan). The Kubelka–Munk theory for multiple scattering [3] was applied to obtain the internal transmittance (Ti), directly correlated with the transparency of the films. Microstructural analysis of cross-sections of the films was carried out by using SEM technique in a JEOL JSM- 5410 (Japan) electron microscope. Film samples were dried in a dessicator by using \( \text{P}_2\text{O}_5 \) (Panreac Química, S.A.). Next, pieces of 6 x 1 mm were cut from films and mounted on copper stubs using double side adhesive tape. Samples were gold coated and observed, using an accelerating voltage of 10 kV.

Film gloss was measured on the free film surface during film drying, at 20, 60 and 85° incidence angles, according to the ASTM standard D523 method [4] using a flat surface gloss meter (Multi.Gloss 268, Minolta, Germany). Measurements were taken in triplicate for each sample and at least four films of each formulation were considered. All results are expressed as gloss units, relative to a highly polished surface of standard black glass with a value close to 100.

The surface morphology of the films was analyzed by using AFM with a Nanoscope III.a, Scanning Probe Microscope (Digital Instruments, Inc. Santa Barbara, CA) with a 125 × 125 µm scan size and a 6 µm vertical range. Measurements were taken from several areas of film surface (50 × 50 µm) using the tapping mode. Water was completely removed from samples, by equilibrating them with \( \text{P}_2\text{O}_5 \) (Panreac Química, S.A.) for two weeks. The following statistical parameters related with sample roughness were calculated according to the method ASME B46.1 [5]: average roughness (Ra: average of the absolute value of the height deviations from a mean surface), root-mean-square roughness (Rq: root-mean-square average of height deviations taken from the mean data plane), and factor of roughness (r: ratio between the three-dimensional surface and two-dimensional area projected onto the threshold plane).

A statistical analysis of data was performed through analysis of variance (ANOVA) using Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, Md.). Fisher’s least significant difference (LSD) procedure was used.

3. RESULTS & DISCUSSION

Lipid materials are frequently added to hydrophilic edible films in order to improve moisture barrier properties. Nevertheless, these hydrophobic materials can affect other important properties of films such as transparency or gloss. Fabra et al. (2009) [1] reported that transparency and gloss are paramount to the film suitability as food coating, due to the impact of such properties on the appearance of the coated products.

According to Kubelka-Munk theory, high values of Ti are associated with transparent films whereas low values of mentioned parameter correspond with more opaque films. Figure 1 shows the spectral distribution of Ti between 400 and 700 nm for the different films. Over the wavelength range considered, a similar pattern was observed for all the samples. Pure starch film was the most transparent in comparison with lipid containing films since it showed the highest values of Ti. The transparency associated with lipid incorporation is due to the introduction of discontinuities (lipid particles) in the hydrocolloid matrix which implies
heterogeneities in the refractive index of the material. The decrease in the transparency of edible films is observed frequently when hydrophobic materials are added to protein [1] or polysaccharide matrices [2]. As concerns to films containing lipids, saturated fatty acids gave rise to more transparent films than oleic acid. This fact can be due to a better dispersion of saturated fatty acids in the starch matrix in comparison with oleic acid. This better dispersion of saturated fatty acids in the hydrocolloid matrix may be related with the interaction of hydrophobic chains of fatty acids with different fractions of starch. Raphaelides et al. (2011) [6] found that amylose–fatty acid interactions took place during extrusion cooking. These interactions could be developed during the homogenization process at high temperature. Nevertheless, oleic acid was not as well dispersed in the polymer matrix, as can be observed in the microstructure analysis, despite the fact that in other polymer networks such as calcium and sodium caseinate [7] oleic acid promoted transparency and was very finely distributed in the polymer network.

![Figure 1. Spectral distribution of internal transmittance (Ti) of studied films.](image1)

Figure 1. Spectral distribution of internal transmittance (Ti) of studied films.

Figure 2 shows the SEM images obtained from the cross-sections of the studied films. Film without lipid showed a very homogeneous appearance in agreement with its highest Ti values. As concerns films containing saturated fatty acids a less smooth, but quite homogeneous, aspect was observed and no lipid aggregates can be clearly distinguished.

![Figure 2. SEM micrographs of the cross-sections of the films.](image2)

Figure 2. SEM micrographs of the cross-sections of the films.
The above mentioned favourable interactions between fatty acids and starch can be the reason of this more fine structural arrangement observed in the SEM images. These lipids formed layered structures in sodium caseinate [1] and hydroxypropylmethylcellulose [2] based films that were not found in these starch based films. However, oleic acid films showed visible droplets, which points to a poor dispersion in the matrix, which agrees with the observed lower transparency of these films. So less affinity between oleic acid and starch polymer chains can be deduced as phase separation occurred to a greater extent.

Film gloss was also affected by lipid addition and the type of fatty acid. Table 1 shows the average gloss values for films with and without fatty acids. This parameter was measured at 20º, 60º and 85º incidence angles and significant differences were observed.

Table 1. Gloss of the films. Mean values (standard deviation).

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<th>20º</th>
<th>60º</th>
<th>85º</th>
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<tr>
<td>Starch-Gly</td>
<td>4.6 (1.6) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>71 (5) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>70 (12) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Starch-Gly-PA</td>
<td>9.7 (2.4) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>56 (10) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>68 (12) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Starch-Gly-SA</td>
<td>23.7 (6.8) &lt;sup&gt;c&lt;/sup&gt;</td>
<td>73 (8) &lt;sup&gt;c&lt;/sup&gt;</td>
<td>83 (10) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Starch-Gly-OA</td>
<td>4.3 (1.3) &lt;sup&gt;d&lt;/sup&gt;</td>
<td>58 (5) &lt;sup&gt;d&lt;/sup&gt;</td>
<td>79 (5) &lt;sup&gt;a&lt;/sup&gt;</td>
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Different superscripts within a column indicate significant differences among formulations (p < 0.05).

Films were reasonably glossy as gloss values at 60º are in order of 60 and, although the film gloss ranking change as a function of the incidence angle, those containing stearic acid showed the highest gloss values. The influence of saturated fatty acids on this parameter is highly dependent of the matrix, but in general, lipid incorporation reduces significantly the gloss in comparison with films without lipid. This was observed in sodium caseinate [1] and hydroxypropylmethylcellulose [2] based films. In these cases, oleic acid increased the gloss, as compared with saturated fatty acid, which was not observed in the corn starch films due to the greater heterogeneity of the matrix when this unsaturated lipid was added, which also affects the surface characteristics.

AFM images (Figure 3) showed that a smooth surface was achieved in the film containing stearic during film drying, in agreement with the highest gloss values. Film containing palmitic acid gave rise to smoother surface than control film (without lipid) and oleic acid containing film, in agreement with gloss values measured at 20º.
Figure 4 shows the roughness parameters obtained from AFM images (three images were analyzed in each case). According to these parameters, films containing saturated fatty acids were the least roughs. Roughness parameters showed that film without lipid and that containing oleic acid behave differently, depending on the parameter considered. Ra and Rq values showed that oleic acid film was the roughest, but r value did not agree with this, showing the lipid free as the roughest. This disappointment was also observed in a previous work [2] for HPMC films. Ra and Rq showed that oleic acid film is the roughest film, as mentioned before, which coincides with that observed SEM and AFM images.

4. CONCLUSION
Incorporation of fatty acids to starch films is an interesting alternative to increase the hydrophobicity of the matrix due to their less polar nature. This does not affect negatively the optical properties of starch films in the case of saturated fatty acid but reduce gloss and transparency when oleic acid was used. Interactions between starch polymer chains and the linear fatty acid chain could contribute to improve the compounds miscibility giving rise to more homogeneous structural arrangement of the compounds thus enhancing the film gloss and transparency. Characterization of other physical properties and thermal behaviour of starch:glycerol:fatty acid films is necessary to complete the analysis of the advantages of this kind composite films.

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REFERENCES