Research note

Chitosan/corn starch blend films with extract from Brassica oleracea (red cabbage) as a visual indicator of fish deterioration

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A B S T R A C T

Chitosan and Starch are polymers that can be obtained from renewable sources, with good film-forming properties and many applications in food industry. So far, one of their most noticeable applications is the development of antioxidant coatings, shelf-life extension films and active packages (Kanatt, Rao, Chawla, & Sharma, 2012; Silva-Weiss, Bifani, Ihl, Sobral, & Gómez-Guillén, 2013; Siripatrawan & Noipha, 2012; Zhang, Lu, & Chen, 2014). In this context, intelligent packages can monitor the conditions of the food in real time. In order to safely indicate the food quality to the consumer, they should be simple, sensitive and efficient. In many food products, changes in pH are an indicator of food spoilage and can visually indicate to the consumer any food quality changes. Therefore, systems that can monitor, detect and visually indicate pH changes in food were developed, including indicators of pH changes in fish (Kuswandi, Restyana, Abdullah, Heng, & Ahmad, 2012; Nopwinyuwong, Trevanich, & Suppakul, 2010; Yoshida, Maciel, Mendonça, & Franco, 2014; Zhang et al., 2014). Fish is among the most consumed foods in the world and is very prone to microbial spoilage, which cause an increase in the pH of fish, due to an increase in volatile nitrogen bases concentration levels (Pacquit et al., 2007). Thus, an inexpensive, accurate, simple and reliable pH-based system that visually indicates fish spoilage can be developed. Although such systems have been developed in recent years (Heising, Dekker, Bartels, & van Boekel, 2012; Kuswandi et al., 2012; Pacquit et al., 2007; Zaragoza et al., 2012), they are not widely available and the literature on the subject is still limited. Furthermore, to the best of our knowledge, fish spoilage monitoring systems that are fully based on renewable sources have not yet been developed. Hence, we report here a system for fish pH monitoring based on chitosan, corn starch and red cabbage extract, which has been successfully applied as a natural pH indicator (Chigurupati, Saiki, Gayser, & Dash, 2002; Mohd, Khan, & Farooqui, 2011).
Moreover, all used materials are inexpensively obtained from renewable sources, as well as having the advantage of being biocompatible and non-toxic, which is of great importance in the food industry.

2. Material and methods

2.1. Extract preparation

Red Cabbage extract was prepared according to Fuleki and Francis (Fuleki & Francis, 1968), with modifications. A sample of approximately 150.0 g of red cabbage was crushed and macerated with 80 mL of ethanol-water (7:3). The pH of the sample was adjusted to 2.0 with HCl (1 mol/L). Subsequently, the material was stored for 24 h at 5 °C, protected from light. After this period, the material was filtered and the extract was centrifuged at 103 × g for 10 min. The supernatant was filtered on Whatman paper #1 and the resulting extract was neutralized to pH 7.0 with NaOH 2.5 mol/L.

2.2. Preparation of starch and chitosan film-forming solutions

For the preparation of corn starch film forming solution, a 50 g/L corn starch aqueous solution (100 mL) was stirred at 70–80 °C until the solution became transparent. Chitosan film-forming solution was prepared dissolving 1 g chitosan (Sigma–Aldrich, St. Louis, MO, USA) in 100 mL of aqueous acetic acid solution (1 mL/100 mL), under stirring during 24 h at room temperature (25 °C).

2.3. Preparation of pH indicator film

The final Chitosan/Starch/Extract film (TII hydrogel) was prepared with the casting technique at a Chitosan/Starch film-forming solution ratio of 3:2 (mL/mL), with incorporation of the extract. The final concentration of the extract in the film was established in 5 mL/100 mL of hydrogel mixture. 1 mL of a 10 g/L sodium tripolyphosphate (Na5P3O10) solution was added to the final hydrogel, in order to promote cross-linking between Chitosan and Starch. The hydrogel was casted (50 ml) in Petri (60 mm diameter) plates and then placed in an oven for 96 h at 35 °C for solvent drying, resulting in the final pH indicator films.

2.4. FT-IR spectroscopy and TG-DSC analysis

FT-IR spectra were obtained in a PerkinElmer spectrophotometer (Spectrum 100), with resolution of 4 cm⁻¹, operating in the range of 4000–600 cm⁻¹ and attenuated reflectance (ATR) Fourier transform. TG-DSC was performed in a Mettler Toledo equipment model TG/DSC-1, using α-Al2O3 crucible (70 µL), with a sample mass of approximately 5 mg, heating rate 20 °C min⁻¹, with dry air flow of 60 mL min⁻¹ and a temperature range of 30–1000 °C.

2.5. Water Vapour Permeability (WVP)

Water vapour permeability rate was determined with the ASTM E 96-95 methodology, as described by Veiga-Santos (Veiga-Santos et al., 2011).

2.6. Light Microscopy (LM) and image texture analysis (GLCM and SDBC)

The microstructures from the surfaces of the films were characterized with Light Microscopy (LM) and Image Texture Analysis. Light microscopy images of the films were captured using a Nikon Eclipse-Ci high-resolution fluorescence microscope at four different magnifications (4×, 10×, 20× and 40×). For all magnifications, film sections of 2 cm² were placed in the microscope base and images from five different areas were taken from each film. In order to conduct the image texture analysis, the obtained images were submitted to texture analysis with the ImageJ software (Abramoff, Magalhaes, & Ram, 2004), using the GLCM (Gray-Level Co-occurrence Matrix) and SDBC (Shifting Differential Box Counting) plug-ins, according to the method purposed by Arzate-Vázquez et al. (2012). In this study three textural features were calculated (Contrast, Homogeneity Index and Entropy). Contrast, that can be used to measure the variance of the image, is a local variation of the grayscale values of a group of pixels. Homogeneity Index represents the local homogeneity of the image. Entropy is an indicator of the complexity of the image, the higher the entropy, the more complex is the image. In order to apply image texture analysis, all the images were converted from RGB to greyscale images.

2.7. Colourimetric analysis

The film colour parameters were determined with a MiniScan EZ Hunterlab spectrophotometer (wavelength range from 400 to 700 nm) at room temperature using the CIELab method, as previously described (Kreyenscheidt, Christiansen, Hübner, Raab, & Petersen, 2010).

2.8. Application as fish deterioration indicator

In order to evaluate the application of the film as fish deterioration indicator, fish fillets acquired in local market were cut into slices measuring 4 cm², and wrapped with the pH indicator film and placed in a 60 mm culture plate. three samples were kept under refrigeration temperature (4–7 °C) and three under room temperature (25 °C). The pH change in the headspace of the samples was monitored during 72 h, in order to evaluate colour change.

3. Results and discussion

3.1. FT-IR spectroscopy

FT-IR is a very powerful tool for detecting interaction in polymeric blends, so in this study, FT-IR was employed to examine the

Fig. 1. FT-IR spectra of Chitosan–Starch (——) film, Chitosan–Starch–Extract (——) film and of red cabbage extract (——).
possible interactions between the film components, as well as the type of interactions. Fig. 1 shows the FT-IR spectra obtained from one sample of pure Chitosan/Starch film (CS) and Chitosan/Starch/Extract ternary film (CSE). Except for the region between 1650 and 1500 cm\(^{-1}\), the spectra of both films are quite identical. CS spectrum shows a typical amide I (C=O) stretching band at 1637 cm\(^{-1}\), while at 1559 cm\(^{-1}\) there is a C–N (amide II) stretching band. FT-IR spectrum for red cabbage extract (Fig. 1) shows a strong absorption band with a maximum at 1028 cm\(^{-1}\) corresponding to aromatic rings, indicating the presence of aromatic compounds in the extract. CSE spectrum shows an inversion in the intensity of the N (amide II) stretching band. FT-IR spectrum for red cabbage extract (Fig. 1) shows a strong absorption band at 1550 cm\(^{-1}\) corresponding to stretching vibration of C\(_6\)O aromatic rings, indicating the presence of aromatic compounds in the extract. CSE spectrum shows a typical amide I (C\(_\text{aromatic}\)=O) stretching band with a maximum at 1028 cm\(^{-1}\), while at 1559 cm\(^{-1}\) there is a C–N (amide II) stretching band. FT-IR spectrum for red cabbage extract (Fig. 1) shows a strong absorption band with a maximum at 1028 cm\(^{-1}\) corresponding to aromatic rings, indicating the presence of aromatic compounds in the extract. CSE spectrum shows an inversion in the intensity of the band at 1634 cm\(^{-1}\) and another at 1550 cm\(^{-1}\) corresponding to stretching vibration of C\(_6\)=C aromatic rings, indicating the presence of aromatic compounds in the extract. CSE spectrum shows an inversion in the intensity of the bands at 1637 cm\(^{-1}\) and 1559 cm\(^{-1}\), indicating van der waals interaction between the amide functionality of chitosan and the red cabbage extract (Fig. 1). Therefore, considering CS spectrum in comparison to CSE (Fig. 1), changes are present in the range between 1500 and 1600 cm\(^{-1}\). These results show that the extract was physically incorporated into the polymeric matrix, meaning that the chemical properties of the extract are not affected by the incorporation into the matrix. It is important, since a chemical interaction between the polymeric matrix and the extract could affect the sensitivity of the extract to pH changes.

### 3.2. TG/DSC

The thermal degradation profiles of the developed films are useful to determine the thermal stability of its components. TG-DSC curve (Fig. 2a) shows the thermal degradation of CS film. The degradation of the film occurs in three stages of mass loss, the first between approximately 51.82 and 149.8 \(^{\circ}\)C, due to loss of adsorbed water (10%). The second mass loss, about 50%, occurs between 200.58 and 352.71 \(^{\circ}\)C, while the third step occurs between 372.0 and 604.7 \(^{\circ}\)C. The DSC curve shows an endothermic peak at 90.2 \(^{\circ}\)C corresponding to loss of adsorbed water, the DSC also shows an exothermic peak at 77 \(^{\circ}\)C and a exotherm between 64.5 and 145.2 \(^{\circ}\)C due to film degradation. TG-DSC curve of CSE (Fig. 2b) shows a 11% mass loss in the range of 50.8–154.0 \(^{\circ}\)C (water), while the second loss of mass happens between 250 \(^{\circ}\)C and \(^{\circ}\)C 322.3 and third between 400.5 and 6970 \(^{\circ}\)C. Comparing both TG-DSC curves, there is similarity in the mass loss of films. However, CSE film has a higher mass loss at a lower temperature than the reference film (CS), despite the minimal variation. This behaviour can be explained due to the composition of the CSE film, which has incorporated red cabbage extract, indicating that the extract has a low thermal stability. Furthermore, the results indicate that both films have thermal stability until the temperature of 50 \(^{\circ}\)C, thus having thermal stability to be safely applied in the food industry.

#### 3.3. Water vapour permeability

Table 1 and Fig. 3 show the results for Water Vapour Permeability (WVP). CS film showed a WVP rate of 1.53 (gh\(^{-1}\) mm m\(^{-2}\) kPa), remaining constant after 48 h of testing, while CSE film showed a WVP rate of 1.60 (gh\(^{-1}\) mm m\(^{-2}\) kPa), also remaining constant after 48 h of testing, both under a humidity of 71% (Fig. 3). The results show that the incorporating of extract does not significantly change the water vapour permeability of the

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>CS mass (g)</th>
<th>CSE mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.24 ± 0.06</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>12</td>
<td>0.26 ± 0.06</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>24</td>
<td>0.26 ± 0.06</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>36</td>
<td>0.27 ± 0.06</td>
<td>0.26 ± 0.05</td>
</tr>
<tr>
<td>48</td>
<td>0.27 ± 0.06</td>
<td>0.26 ± 0.05</td>
</tr>
<tr>
<td>60</td>
<td>0.27 ± 0.06</td>
<td>0.26 ± 0.05</td>
</tr>
<tr>
<td>72</td>
<td>0.27 ± 0.06</td>
<td>0.26 ± 0.05</td>
</tr>
<tr>
<td>84</td>
<td>0.27 ± 0.06</td>
<td>0.26 ± 0.05</td>
</tr>
<tr>
<td>96</td>
<td>0.27 ± 0.06</td>
<td>0.26 ± 0.05</td>
</tr>
</tbody>
</table>

±SD Mean deviation of five samples.
film. Furthermore, WVP values are in the range of WVP obtained for similar blend polymers (Mathew & Abraham, 2008; Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009). Although the values for WVP is similar to the values obtained for other biodegradable films, they are far from those from petroleum-based polymers (Debeaufort & Voilley, 1994; Metz, Vandeven, Potreck, Mulder, & Wessling, 2005), which indicates that chitosan–starch films still need further improvements, since a good polymer for food packaging applications should have a WVP value comparable to that of widely used petroleum-based polymers, for example such as low-density polyethylene, which has a value comparable to that of widely used petroleum-based polymer for food packaging applications should have a WVP rate of 9.14 × 10⁻¹⁵ m² Pa⁻¹ s⁻¹ (Garcia, Pinotti, Martino, & Zaritzky, 2004).

3.4. Light microscopy and image texture analysis (GLCM and SDBC)

Fig. 4 shows the LM images of the films at all magnifications. LM images of CSE films are smoother and more homogeneous at all magnifications, when compared to CS film images. At higher magnifications, it can be observed some irregularities in all images of film surfaces. These irregularities can be assigned to the agglomeration of starch molecules, which is prone to agglomeration (Silva-Weiss et al., 2013). Moreover, air bubbles and holes in the films were not observed. In order to corroborate the description of film structure observed with LM, LM images were mathematically treated with GLCM and SDBC algorithms, in order to obtain their textural features. Thus, the values of textural features obtained from LM images with GLCM and SDBC algorithms are listed in Table 2. The image textural features are in accordance with LM, LM images have a higher homogeneity index in all magnifications, when compared to CS film images. CSE film images have a lower entropy value and a lower contrast value, which indicates a uniform texture, when compared to CS film images. The smoother and homogeneous images of CSE film indicate that this film has a more homogeneous surface when compared to CS film. Moreover, this indicates that the interaction of red cabbage extract with chitosan and starch molecules allows the formation of a continuous polymeric matrix.

### Table 2

<table>
<thead>
<tr>
<th>Magnification</th>
<th>Contrast</th>
<th>Homogeneity index</th>
<th>Entropy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CS</td>
<td>CSE</td>
<td>CS</td>
</tr>
<tr>
<td>4x</td>
<td>561.74 ± 0.01</td>
<td>317.32 ± 0.01</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>10x</td>
<td>406.81 ± 0.10</td>
<td>371.62 ± 0.01</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td>20x</td>
<td>406.34 ± 0.80</td>
<td>366.00 ± 0.02</td>
<td>0.08 ± 0.05</td>
</tr>
<tr>
<td>40x</td>
<td>132.16 ± 0.02</td>
<td>95.34 ± 0.54</td>
<td>0.13 ± 0.01</td>
</tr>
</tbody>
</table>

±SD Mean deviation of five samples.

3.5. Colourimetric analysis

Colour parameters were determined at a pH range of 2–13 (Table 3). According to CIELab colour system, the scale for parameter $L^*$ is between 100 (white) to 0 (black), while for parameters $a^*$ and $b^*$ are respectively $+a$ (red) to $-a$ (green), $+b$ (yellow) to $-b$ (blue). According to Table 3, for the parameter $a^*$, it is observed that this pattern has high values at pH 7 (−0.49), 8 (−0.47) and 9 (−0.01), while at pH 12 (−6.00) and 13 (−1.49) the values are lower. This means that the green colour has greater intensity at higher pH values. At pH 2 to 4, positive values are present for parameter $a^*$, this indicating presence of red colour. Parameter $b^*$ has positive values at all pH ranges, which means that the yellow colour is present in lesser or greater intensity at all pH values. The colour variation ($ΔE^*$) was larger at pH 2 (14.03), 4 (7.28), as well as at pH 12 (7.52) and 13 (9.61). Hence, the film can have good colour variation, depending on the pH value, which indicates good visual colour variability.

3.6. Application as fish deterioration indicator

At room temperature (25 °C) and refrigeration temperature (4–7 °C), the indicator film (CSE) was initially transparent. At room temperature, for 12 h no colour change was visible in the indicator film, after 16 h the colour began to change to blue, indicating fish pH increase and initial spoilage, and after 72 h the colour completely changed to yellow thus indicating complete fish spoilage. At refrigeration temperature, no colour change was observed during 72 h, but after this time, the colour changed to light blue and to yellow after 7 days, indicating fish spoilage. Therefore, the film can detect fish pH changes, which is an indicative of spoilage. Although other tests,
such as microbial spoilage tests, are needed to validate fish spoilage and test the limitations of this system. It is accepted in the literature that pH changes in fish and test the limitations of this system, it is accepted in the literature.

### Table 3

<table>
<thead>
<tr>
<th>pH</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>84.50 ± 0.03</td>
<td>13.97 ± 0.06</td>
<td>3.22 ± 0.01</td>
<td>14.03</td>
</tr>
<tr>
<td>3</td>
<td>89.91 ± 0.15</td>
<td>3.82 ± 0.22</td>
<td>7.47 ± 0.06</td>
<td>6.25</td>
</tr>
<tr>
<td>4</td>
<td>84.50 ± 0.10</td>
<td>7.20 ± 0.03</td>
<td>5.96 ± 0.02</td>
<td>7.28</td>
</tr>
<tr>
<td>5</td>
<td>86.28 ± 0.02</td>
<td>3.34 ± 0.02</td>
<td>6.84 ± 0.01</td>
<td>4.55</td>
</tr>
<tr>
<td>6</td>
<td>90.38 ± 0.06</td>
<td>0.75 ± 0.01</td>
<td>4.78 ± 0.01</td>
<td>3.97</td>
</tr>
<tr>
<td>7</td>
<td>90.72 ± 0.20</td>
<td>0.49 ± 0.01</td>
<td>5.48 ± 0.06</td>
<td>4.49</td>
</tr>
<tr>
<td>8</td>
<td>91.81 ± 0.03</td>
<td>0.47 ± 0.01</td>
<td>5.41 ± 0.09</td>
<td>5.51</td>
</tr>
<tr>
<td>9</td>
<td>89.35 ± 0.08</td>
<td>0.01 ± 0.01</td>
<td>6.04 ± 0.02</td>
<td>3.66</td>
</tr>
<tr>
<td>10</td>
<td>92.13 ± 0.02</td>
<td>1.71 ± 0.01</td>
<td>3.79 ± 0.01</td>
<td>5.47</td>
</tr>
<tr>
<td>11</td>
<td>88.76 ± 0.01</td>
<td>1.12 ± 0.01</td>
<td>5.10 ± 0.01</td>
<td>2.77</td>
</tr>
<tr>
<td>12</td>
<td>82.08 ± 0.01</td>
<td>0.60 ± 0.02</td>
<td>8.03 ± 0.04</td>
<td>7.52</td>
</tr>
<tr>
<td>13</td>
<td>92.70 ± 0.03</td>
<td>1.49 ± 0.01</td>
<td>10.94 ± 0.05</td>
<td>9.61</td>
</tr>
</tbody>
</table>

±SD Mean deviation of a triplicate.

4. Conclusion

The results present in this study show that a very sensitive visual pH indicator film based on renewable sources has been developed. The results also show that the film components do not react with each other and that the blend has good thermal stability. Furthermore, the indicator film has a uniform morphology and continuous polymeric matrix, with few irregularities. The indicator film also has good response to pH variation, safely detecting pH changes. Thus, it has potential to be used as a visual indicator of food deterioration. Future developments include the study of the film response in various temperatures, the study of extract stability in the film, as well as the study of the mechanical properties of the film. These should provide a clear picture on the real potential use of this system as part of a smart food packaging.

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