Effect of Protein and Sorbitol Concentrations on the Properties of Fish Gelatin Films

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Abstract

Edible films were prepared with bone gelatins extracted from red snapper and grouper at different concentrations of protein (1, 2, 3, 4 and 5%) and sorbitol (10, 20, 30, 40 and 50% of protein). Gelatin films made with 5% protein had maximum tensile strength (TS), elongation at break (EAB) and Young’s modulus (YM) in the range of 63.2 to 73.1 MPa, 67.0 to 76.1% and 724 to 809 MPa, respectively. Gelatin films prepared with increasing concentration of sorbitol had reduced the TS and YM of the films, but increased the EAB from 21.7 to 63.7% in grouper and 27.3 to 67.3% in red snapper. Grouper gelatin films had greater TS, YM and thickness compared to red snapper gelatin films. Opacity of the films increased with protein and sorbitol concentrations even in the UV region, which would make them excellent light barriers. Grouper gelatin films had high strength, while red snapper films had high elongation.

Keywords: Fish gelatin; Sorbitol; Bone gelatin; Edible films; Tensile strength

1. Introduction

In recent years, biodegradable edible films from natural biological materials are gaining importance. Among the natural materials, protein based edible films are the most appealing for their nutritional value and enhancement of the quality and safety of food products (Gennadios et al., 1997). Gelatin is a thermally denatured protein obtained from collagen by acidic or alkaline processes (Arvanitoyannis and Biliaderis, 1998) and instead of that has been extensively studied for its film-forming capacity and applicability as an edible film to protect food against drying, light and oxygen. It has been traditionally produced through hydrolysis of bones and skin from bovines and porcines, and this instead of which often creates difficulties for people with dietary restrictions.
and due to transmission of animal diseases like bovine spongiform encephalopathy (Sobral et al., 2001; Gilsenan and Ross-Murphy, 2000). To overcome these difficulties, gelatin from marine fish is considered as an alternative to animal gelatin as well as synthetic plastics for making edible films (Gomez-Guillen et al., 2002). Fish gelatin has been extracted from several fish species (Zhou et al., 2006; Yang and Paulson, 2000; Cheow et al., 2007; Jeyashakila et al., 2012a) through various extraction procedures.

Gelatin films are generally formed using several chemical plasticizers like sorbitol, glycerol, ethylene glycol, sucrose, polyethylene glycol, glutaraldehyde, genipin, tannic acid and ferulic acid (Bigi et al., 2000; Cao et al., 2007). Plasticizers are added during gelatin film formation to reduce the protein–protein interaction resulting in an increased mobility of protein molecules (Gontard et al., 1993; Banker and Irissin, 1996). Addition of the plasticizer influences gas and water permeability of films. So, they must be added in certain concentrations to obtain the films with improved flexibility without losing the barrier properties (Sothornvit and Krochta, 2000). The difference in composition, size, structure and shape of plasticizers also directly influence the ability to form the film network (Orliac et al., 2003).

Although gelatin films are prepared using several plasticizers, Thomazine et al. (2005) reported that gelatin films made with glycerol had low mechanical properties and high water sensitivity than the films made with sorbitol. Film formation using different concentrations of protein and plasticizer has been standardized by few workers but mainly using fish skin gelatin (Jongjareonrak et al., 2006; Limpisophon et al., 2009). There is no information available on the formation of films from fish bone gelatin and hence, this study was undertaken to standardize the concentrations of protein and the plasticizer, sorbitol in the formation of films with better mechanical and barrier properties.

2. Materials and Methods

2.1. Raw materials

Red snapper (Lutjanus campechanus) and grouper (Epinephelus lanceolatus) are two commercially important marine fin fishes caught along the East Coast of Tamilnadu, India having an average catch of 28,000 tonnes per year. The average lengths and weights of red snapper were 55 cm and 5 kg; while that of grouper were 45 cm and 3 kg, respectively. They are mainly processed in fillet form and exported to European countries by the fish processing industries. Filleting process generates about 55-60% as processing wastes, mainly fish frames. Fish frames consist of bones and other superfluous materials, excluding head region. They were obtained from a private fish processing plant, M/s Kondia Seafoods Pvt. Ltd, Tuticorin, South India and brought to the laboratory in insulated containers. The superfluous materials attached to the fish frames were removed manually to separate the bones. Bones were then cut into small pieces using sharp knives, washed with potable water, packed in polyethylene bags and held at -20°C, and used for film formation within a month.

2.2. Extraction of gelatin

Gelatin extraction was carried out as per the earlier described method (Jeyashakila et al., 2012a). Bones were treated twice with 0.2% NaOH at 1:6 ratio (w/v) for 45 min to remove the non-collagenous protein. They were washed thoroughly with water and treated twice with 0.2% H₂SO₄

at 1:6 ratio (w/v) for 45 min, to increase swelling as well as to remove salts. They were then washed thoroughly with water and treated twice with 1% citric acid at 1:6 ratio (w/v) for 45 min; and again washed with water thoroughly. The final extraction was carried out with distilled water at 1:1 ratio (w/v) at 45°C for 24 h. The extract was filtered through Whatman No. 4 filter paper (Whatman International Ltd, Maidstone, England) under vacuum, and lyophilized in a lyophilizer (Alpha 2, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). The protein content of the dried gelatin was determined by Kjeldahl method using a Kel plus Nitrogen analyzer (Pelican Equipments, Chennai, India) following AOAC method (1995). The pH of extracted gelatin was measured using pH meter at 25°C by dissolving the gelatin, 1.0 g in 100 ml distilled water (Cheow et al.,2007) and the values were 5.18 and 5.65 for red snapper and grouper, respectively.

2.3. Formation of gelatin films
Gelatin film forming solution (FFS) was prepared by mixing lyophilized gelatin powder in distilled water to obtain the final protein concentrations of 1, 2, 3, 4 and 5% (w/v) with sorbitol as a plasticizer (30% of the protein) to standardize protein concentration. Similarly, gelatin FFS was prepared with 3% protein concentration containing plasticizer at the concentrations of 10, 20, 30, 40 and 50% to standardize sorbitol concentration. The FFS (15 ml) was stirred well for 30 min, filtered and cast on the circular polypropylene plates 63 cm², (Laxpro Pvt Ltd, Chennai, India) and dried at ambient temperature (30°C) for 14-18 h.

2.4. Mechanical properties
Tensile strength (TS), elongation at break (EAB) and Young’s modulus (YM) were determined as per the ASTM D 882 methods (ASTM, 2002) using a Universal Testing Machine (TA plus Texture Analyzer, Lloyd instruments, West Sussex, U.K). Films were cut into rectangles of 25 x 70 mm size and fixed on the grips of the device with a gap of 30 mm. They were then pulled apart at a crosshead speed of 20 mm/s and a preload of 2 N. The TS was calculated by dividing the maximum force at break by the original cross sectional area of the film, and expressed in MPa. The EAB and YM were calculated based on the length extended and ratio of stress to strain of the films and expressed in % and MPa, respectively.

2.5. Film thickness
Thickness of the gelatin films were measured to the nearest 5 µm with the laboratory micrometer screw gauge (Labtech International, Ambala, Haryana, India) at six random positions as described by Jeyashakila et al. (2012b).

2.6. Opacity
Barrier properties of gelatin films against ultraviolet (UV) and visible light were measured at selected wavelength between 200 and 800 nm using a UV–Visible Spectrophotometer (Model V-530, Jasco, Kyoto, Japan) according to the method described by Limpisophon et al.(2009). Films were cut into a rectangular piece of 12 x 43 mm size and directly placed in a spectrophotometer test cell made of quartz with >80% transmission, using the empty cell as the reference. Opacity index of the films was calculated using the following equation. Opacity (%) =100 % - T, where, T = Transmittance (%) at each wavelength.
2.7. Light transmission
Transparency of gelatin films was measured using the UV/Vis Spectrophotometer according to the method of Gomez-Guillen et al. (2007). Transparency was calculated using the equation, \( T = \frac{Abs_{600}}{x} \), where, \( Abs_{600} \) is the value of absorbance at 600nm and \( x \) is the film thickness (mm). According to this equation, higher values of \( T \) indicate lower degrees of transparency.

2.8. Water vapor transmission rate (WVTR)
WVTR of the films was measured according to the standard ASTM method (1987) as described by Jeyashakila et al. (2012b). Gelatin film was cut into 90 x 90mm pieces and each piece was put onto a permeability cup. The cup was previously filled with fused calcium chloride. The cup was then sealed with a cover and put into a humidity chamber (Krishna Scientific Supplies, Chennai, India) at 25°C and 90% RH for 24 h. The weight of the sealed cup was measured at the beginning and after 1 h interval. WVTR was calculated using the following equation

\[
WVTR = \frac{10,000 \times Q}{A} \text{ g/m}^2/\text{day} @ 90\% \text{ RH at 25 }^\circ\text{C}
\]

where, \( Q = \) Quantity of water vapor passed through the test material; \( A = \) Area of test material

2.9. Statistical analysis
Average mean values were calculated from three determinations and standard deviations were determined. The data were subjected to analysis of variance (ANOVA) using SPSS package (SPSS 11.0 for windows, SPSS Inc. Chicago, IL) to find out the significant differences among the different properties of the gelatin films with respect to different protein and sorbitol concentrations.

3. Results and Discussion

3.1. Effect of protein concentrations on gelatin film formation
TS and EAB of the films are the two important properties of packaging materials. Bone gelatin films prepared from both the fish species showed different TS, EAB and YM at different concentrations of protein (Table 1). Films prepared with 1% protein were too thin to peel off, while with 5% protein were thick and could be easily peeled off. In general, the TS of the bone gelatin films increased with the increasing protein concentration and the increase was significant between each concentration of protein (p<0.05). The increase in the number of protein chains per unit surface at higher concentrations had led to an increase in the number of potential intermolecular interactions contributing to higher TS (Cuq et al., 1996). Films made with grouper bone gelatin had high TS than those made from red snapper, probably due to differences in the size of protein chains as well as amino acid composition as observed earlier by Muyonga et al.(2004) between bigeye red snapper and brownstripe red snapper.

EAB of the films also increased with the increasing protein concentrations. Remarkable increase was noticed between 1 and 2 % as well as 2 and 3 % protein concentration (p<0.01). Higher protein concentration had resulted in a higher aggregation of protein and thus improved the flexibility of films (Jongjareonrak et al., 2006). The same result was also observed with the films made from blue shark (Prionace glauca) skin gelatin (Limpisophon et al., 2009), fish water soluble protein (Iwata et al., 2000) and casein protein (Brault et al., 1997). The increase in TS and EAB of the gelatin films with the increase in protein concentrations was mainly due to more protein chain-to-chain interactions.
interactions and mobility of protein chains (Hoque et al., 2011). The EAB of the films made with red snapper was higher than grouper gelatin. The YM of the fish gelatin films also increased with increasing protein concentrations, particularly the increase was more between 1, 2, and 3% protein concentration. As noticed with the TS of the films, grouper gelatin films were stiffer than red snapper films.

**Table 1** Effect of protein concentrations on properties of gelatin films

<table>
<thead>
<tr>
<th>Protein (%)</th>
<th>TS (MPa)</th>
<th>EAB (%)</th>
<th>YM (MPa)</th>
<th>Thickness (µm)</th>
<th>Transparency (Abs600/x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RsG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.60±1.0</td>
<td>11.8±1.7</td>
<td>154±15</td>
<td>15.9±2.1</td>
<td>7.91±0.1</td>
</tr>
<tr>
<td>2</td>
<td>32.5±1.8</td>
<td>34.0±2.4</td>
<td>360±21</td>
<td>50.6±3.1</td>
<td>6.94±0.4</td>
</tr>
<tr>
<td>3</td>
<td>43.7±3.1</td>
<td>58.7±3.8</td>
<td>510±12</td>
<td>76.3±0.9</td>
<td>6.43±0.1</td>
</tr>
<tr>
<td>4</td>
<td>47.8±2.0</td>
<td>69.4±3.6</td>
<td>586±29</td>
<td>98.0±2.1</td>
<td>5.15±0.7</td>
</tr>
<tr>
<td>5</td>
<td>63.2±1.0</td>
<td>76.1±4.3</td>
<td>724±22</td>
<td>104±3.3</td>
<td>3.08±0.6</td>
</tr>
<tr>
<td>GrG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.80±2.4</td>
<td>13.8±1.9</td>
<td>176±20</td>
<td>16.7±4.6</td>
<td>4.15±0.5</td>
</tr>
<tr>
<td>2</td>
<td>36.3±1.8</td>
<td>27.7±6.2</td>
<td>413±14</td>
<td>54.8±3.4</td>
<td>3.76±0.4</td>
</tr>
<tr>
<td>3</td>
<td>48.6±1.5</td>
<td>51.9±7.6</td>
<td>570±10</td>
<td>82.1±2.0</td>
<td>2.77±0.2</td>
</tr>
<tr>
<td>4</td>
<td>54.9±1.7</td>
<td>63.8±2.7</td>
<td>767±27</td>
<td>99.0±4.0</td>
<td>2.40±0.2</td>
</tr>
<tr>
<td>5</td>
<td>73.1±2.2</td>
<td>67.0±5.3</td>
<td>809±26</td>
<td>112±2.0</td>
<td>1.14±0.7</td>
</tr>
</tbody>
</table>

RsG - red snapper gelatin film, GrG - grouper gelatin film with 30% sorbitol as plasticizer. All values are mean ± standard deviation of triplicate analysis. Different superscript letters a, b, c, d, e in the same column indicate significant differences among the different films for the same species. Different superscript letters A, B, C, D, E in the same column indicate significant differences among the fish species for the same film (P<0.05).

Thickness of the bone gelatin films increased with the increase in the concentration of protein (Table 1). Jongjareonrak et al. (2006) also reported that the thickness of bigeye red snapper and brownstripe red snapper skin gelatin films increased with the increase in the protein concentration from 1 to 5%. Thickness of the films made with grouper gelatin was slightly higher than red snapper gelatin.

Transparency of bone gelatin films decreased with the increasing concentrations of protein (Table 1), while the opacity increased (Fig. 1). The gelatin films made with 5% protein had greater thickness but lower transparency (p<0.05) and they would absorb the light more effectively than those with low protein content and thickness. The same results were also reported by Jongjareonrak et al. (2006) with respect to bigeye red snapper and brownstripe red snapper skin gelatin films. The opacity was high in the UV range than visible light region for the fish gelatin films. The high opacity in the UV range of 200-280 nm indicated that the fish gelatin films can effectively prevent UV light-induced lipid oxidation when applied in food systems (Gomez-Guillen et al., 2007). Limpisophon et al. (2009) stated that opacity of shark gelatin films increased with increasing protein concentrations and this could be due to the increase in the ratio of high molecular-weight proteins that had effectively absorbed the UV light. They also indicated that the shark skin gelatin films exhibited low transparency and high opacity with the increasing protein...
concentration and thus had great light barrier properties. Transparency of films formed with grouper gelatin was lower than those made from red snapper. On the other hand, the opacity of the red snapper gelatin films was slightly higher than the grouper gelatin films in the UV region. The differences in the transparency and opacity among the species could be due to the inherent compositional variations (Jongjareonrak et al., 2006). Therefore, grouper fish gelatin films were found to be superior with better barrier properties than red snapper fish gelatin films.

![Fig 1. Effect of protein concentrations on opacity of gelatin films with 30% sorbitol as plasticizer. RsG - red snapper gelatin film; GrG - grouper gelatin film.](image)

WVTR of gelatin films prepared with different protein concentration are given in the Fig. 3. An increase in the protein concentration had led to decrease in WVTR from 1408 to 805 g/m2/day @ 90% RH at 25 º C. The decrease was significant between different protein concentrations (p<0.05). There was a reduction in WVTR by 74% with the increasing protein concentration from 1-5%. The decrease in WVTR with increasing protein concentration was also reported in gelatin films (Park et al., 2008) and whey proteins films (Fang et al., 2002; Gounga et al., 2007). The decrease in the free volume of the polymer matrix caused by protein-protein chains interactions had reduced the permeability of the gelatin films. Grouper gelatin films had lower WVTR than the red snapper films, irrespective of protein concentrations.

### 3.2. Effect of sorbitol concentrations on gelatin film formation

Mechanical properties of fish bone gelatin films formed using different concentrations of sorbitol are given in Table 2. The TS of the gelatin films significantly decreased with increasing sorbitol concentrations (p<0.05). The TS of red snapper gelatin films decreased from 59.3 to 24.1 MPa, while that of grouper films decreased from 62.4 to 30.2 MPa. Grouper films had higher TS than red snapper films at even the lower concentration of sorbitol. A decrease in TS of whey protein films with the addition of sorbitol or glycerol (McHugh and Krochta, 1994) and gelatin films with the additions of glycerol (Lim et al., 1999) were also reported by few authors. Similarly, a reduction in puncture force of Atlantic sardine myofibrilla protein films (Cuq et al., 1996) with the increasing concentration of glycerol as well as bovine hide and pig skin gelatin films (Sobral et al., 2001) with the increasing concentration of sorbitol were also reported. When plasticizers are incorporated into
the gelatin films, the direct interactions and proximity between proteins chains would get reduced (Jongjareonrak et al., 2006) leading to lower strength of the films.

**Table 2 Effect of sorbitol concentrations on properties of gelatin films**

<table>
<thead>
<tr>
<th>Plasticizer</th>
<th>TS (MPa)</th>
<th>EAB (%)</th>
<th>YM (MPa)</th>
<th>Thickness (µm)</th>
<th>Transparency (Abs600/x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RsG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>59.3±0.8aA</td>
<td>27.3±5.1aA</td>
<td>796±10aA</td>
<td>45.6±1.3aA</td>
<td>7.32±0.2aA</td>
</tr>
<tr>
<td>20</td>
<td>48.7±1.2bB</td>
<td>33.9±4.2bB</td>
<td>605±15bB</td>
<td>61.5±1.9bB</td>
<td>6.74±0.1bB</td>
</tr>
<tr>
<td>30</td>
<td>43.7±3.1cC</td>
<td>58.7±2.3cC</td>
<td>510±12cC</td>
<td>76.3±0.9cC</td>
<td>6.41±0.2bcC</td>
</tr>
<tr>
<td>40</td>
<td>36.1±2.4dD</td>
<td>64.9±3.7dD</td>
<td>390±21dD</td>
<td>92.1±2.1dD</td>
<td>5.16±0.1cdD</td>
</tr>
<tr>
<td>50</td>
<td>24.4±5.1eE</td>
<td>73.3±6.1eE</td>
<td>367±17eE</td>
<td>97.1±1.2eE</td>
<td>4.90±0.1eE</td>
</tr>
<tr>
<td>GrG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>62.4±2.3aA</td>
<td>21.7±2.9aA</td>
<td>785±18aA</td>
<td>47.7±1.0aA</td>
<td>4.65±0.7aB</td>
</tr>
<tr>
<td>20</td>
<td>53.9±1.9bB</td>
<td>29.4±2.6bB</td>
<td>615±12bB</td>
<td>65.7±0.9bB</td>
<td>3.53±0.3bC</td>
</tr>
<tr>
<td>30</td>
<td>48.6±1.5cC</td>
<td>51.9±7.6cC</td>
<td>570±10cC</td>
<td>82.1±2.0cC</td>
<td>2.71±0.1cD</td>
</tr>
<tr>
<td>40</td>
<td>41.6±1.6dD</td>
<td>59.0±2.2dD</td>
<td>487±31dD</td>
<td>96.1±1.3dD</td>
<td>1.47±0.6deD</td>
</tr>
<tr>
<td>50</td>
<td>30.2±4.0eF</td>
<td>63.7±2.3eF</td>
<td>405±25eF</td>
<td>103±2.0eF</td>
<td>1.12±0.1eF</td>
</tr>
</tbody>
</table>

RsG - red snapper gelatin film, GrG - grouper gelatin film with 3% protein concentration. All values are mean ± standard deviation of triplicate analysis. Different superscript letters a, b, c, d, e in the same column indicate significant differences among the different films for the same species. Different superscript letters A, B, C, D, E in the same column indicate significant differences among the fish species for the same film (P<0.05).

On the other hand, the EAB of films increased with increasing sorbitol concentration from 10 to 50%. The EAB increased from 27.3 to 67.3% in red snapper gelatin films and from 21.7 to 63.7% in grouper films. This result was in accordance with Jongjareonrak et al. (2006) who observed an increase in EAB from 24.0 to 95.0% in brown red snapper skin gelatin films and from 3.40 to 50.3% in bigeye red snapper films with the increase in glycerol concentration from 25 to 75% of the protein. Earlier, Gontard et al. (1993) and Mangata et al. (2001) have also found that EAB of wheat gluten films increased from 4 to 22% with the increasing glycerol concentration. The plasticizer could easily form hydrogen bond with protein chains, resulting in a reduction of intermolecular protein-protein interaction and thus, increased the mobility of gelatin leading to the increase in the EAB of films (Gontard et al., 1993). This in turn had led to the increase in the moisture content of films because of the high hygroscopic character of sorbitol (Sobral et al., 2001). The YM of the gelatin films also decreased with the increase in plasticizer concentrations as that of the TS. The differences were more significant between 10 and 20 % as well as 30 and 40 % sorbitol concentrations.

Thickness of the gelatin films also increased with the increase in the sorbitol concentrations (Table 2) similar to protein concentrations. Similar results were also observed with cuttlefish skin gelatin films (Hoque et al., 2011) and pigskin gelatin films (Park et al., 2008). There was not much differences in the thickness of the bone gelatin films prepared from red snapper and grouper (p>0.05). At the higher concentration of sorbitol, the thickness of the grouper gelatin films were high than red snapper films. It has been noted that greater thickness of the films gives a larger cross
sectional area that allows greater elongation of films (Longares et al., 2004). Therefore, the higher EAB of gelatin films could be attributed partially to the differences in the thickness.

Transparency of gelatin films decreased while the opacity increased with the increasing concentration of sorbitol. Transparency decreased from 4.65 to 1.12% in grouper bone gelatin films and from 7.32 to 4.90% in red snapper gelatin films (p<0.05) (Table 2). Jongjareonrak et al. (2006) opined that bone gelatin films can efficiently prevent light if they have very low transmission or more opaque.

Fig.2. Effect of plasticizer concentrations on opacity of gelatin films with 3% protein concentration. RsG - red snapper gelatin film; GrG - grouper gelatin film.

Opacity increased with increasing the sorbitol concentration as well as with decreasing light wave lengths from 800 to 200 nm (p<0.05). Limpisophon et al. (2009) also observed that an increase in the opacity with the increase in plasticizer concentrations with respect to shark gelatin films; and found that it was due to changes in the refractive index between gelatin and plasticizer.

Fig.3. The effect of protein and plasticizer concentrations on WVTR of gelatin films. RsG - red snapper gelatin film; GrG - grouper gelatin film.
The changes in the opacity between the red snapper and grouper bone gelatin films was not very significant (p>0.05) in the UV region, but showed greater differences in the visible region, particularly at wave lengths 350, 400 and 500 nm (p<0.05) (Fig. 2). Grouper films that had lower transparency and higher opacity can provide better light barrier properties than red snapper gelatin films.

WVTR of gelatin films increased with the increase in the plasticizer concentrations (Fig. 3) unlike the protein concentrations. WVTR of the gelatin films showed significant increase (p<0.05) between 40 and 50% sorbitol concentrations. Such increase in WVTR with the increasing plasticizer concentration was also observed in rice starch films (Laohakunjit and Noomhorm, 2004) and chitosan films (Lamim et al., 2006). The hydroscopic nature of the plasticizer had probably led to the increase in the water permeability of gelatin films. Grouper gelatin films had lower WVTR than the red snapper films, irrespective of plasticizer concentrations.

It can be inferred from this study that fish bone gelatin films with good TS and excellent EAB could be prepared at 3-5% protein concentration. Lower transparency and higher opacity provided the bone gelatin films the good light barrier properties particularly in UV region. WVTR for these films can be improved by increasing the protein concentrations and lowering the sorbitol concentrations. Grouper gelatin films were found superior than red snapper films, in terms of mechanical as well as barrier properties. Fish bone gelatin films could be serving as an alternative for synthetic polymer films, as they are obtained from natural sources, biodegradable and edible. However, further studies are required to improve barrier properties and then to scale up the process.

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