Color characteristics and microstructure of bioactive films on various structure formers

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Abstract. The color and transparency of the films in which food products are packaged are one of the important factors for consumers when choosing food products. The aim of this research was to investigate the color characteristics and transparency films made on different biopolymer bases and with the addition of active components. The results of determining color characteristics showed different effects of introducing protein hydrolyzate into alginate, agar and pectin film bases. The films based on pectin have a higher yellowness value. Films based on agar turned out to be the most transparent. The color intensity (Chroma) is most pronounced in films based on pectin with the addition of protein hydrolysate. Thus, the addition of protein hydrolyzate affects the transparency of the films, as well as their color characteristics and color intensity, however, the nature of these changes directly depends on the structure-forming matrix used.

1 Introduction

The main purpose of food packaging is to prevent biochemical and mechanical damage. Over time, packaging has moved from the category of primary containers to a more complex instrument with certain special properties.

Ideal food packaging should not only be economical and convenient to use, but also have excellent barrier properties that protect products from drying out, oxygen and moisture, and also have mechanical strength [1]. Packaging transparency is important to consumers as it allows them to assess the freshness and appearance of a product before purchasing. It is also important that the packaging is made from renewable sources, can be recycled after use and has biodegradable properties [2].

Today, due to the widespread use of plastic and plastic packaging, the level of pollution of the entire planet as a whole, and the World Ocean in particular, is insanely huge. Recently, people have increasingly begun to try to correct this by using natural biopolymers, including: carrageenan, chitosan, starch, agar, and alginate. Biopolymers have attracted the attention of scientists for their advantages, namely chemical stability, low permeability to oxygen, and high biodegradability. All these properties make these biopolymers a potential replacement

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for synthetic polymers, which take a long time to decompose under environmental conditions and subsequently accumulate environmental objects in the form of microplastics [3].

Scientists have noted that environmental friendliness is currently a key ideology. The problem of global environmental pollution with plastic will be solved by modern solutions for the production and use of natural biopolymers from renewable resources.

However, animal proteins and natural polysaccharides, with their specific chemical composition and structure, have both positive and negative properties that are important in the manufacture of edible films [4].

The transparency of film coatings is a key factor in helping consumers make a choice in favor of fresh and high-quality products; therefore, this property must be preserved as much as possible during the production of films. Most often, seaweed processing products are used as the structure-forming the basis of edible films, giving the films flexibility and transparency - carrageenan, sodium alginate, and agar. But other biopolymers also make it possible to obtain transparent films, for example collagen or pectin.

In order to improve the quality of edible films, various fillers are added to them, which can also give the films additional properties, such as antioxidant and antibacterial [5]. However, these additives and fillers can negatively affect the clarity of the resulting films. For example, adding almost any type of oil has a negative effect on this indicator [6].

In addition, the additives and active components used, being integrated into the polymer matrix of the film, can affect the structure of the film and, as a consequence, its mechanical and barrier properties.

The aim of this research was to investigate the color characteristics and transparency films made on different biopolymer bases (sodium alginate, pectin, and agar) and with the addition of active component – protein hydrolyzate.

2 Materials and Methods

2.1. Preparation of Films

The objects of research were films made on various bases with the addition of the plasticizer glycerol and the active component of protein hydrolyzate. The films were prepared as follows:

1. Agar based. 2% agar was added to the heated water and brought to a boil. After uniform distribution and swelling, the agar was cooled to 60°C with constant stirring on a magnetic stirrer, and 3% glycerol, and 1% protein hydrolyzate solution (1:10) were added, then poured into Petri dishes and dried at a temperature of 30°C until completely dry (Ag-PH).

2. Based on alginate. 1.5% alginate was added to water heated to 40°C with constant stirring on a magnetic stirrer. After 20 minutes of stirring, 3% glycerol and 1% protein hydrolyzate solution (1:10) were added, then poured into Petri dishes and dried at a temperature of 30°C until completely dry (Al-PH).

3. Based on pectin. 1.5% pectin was added to hot water with constant stirring on a magnetic stirrer, heated to 70°C and stirred until the pectin was completely dissolved. Then it was cooled to 40°C and 3% glycerol and 1% protein hydrolyzate solution (1:10) were added, then poured into Petri dishes and dried at a temperature of 30°C until completely dry (P-PH).

Control samples were films based on agar, alginate, and pectin (Ag-C, Al-C, and P-C, respectively) without the addition of protein hydrolyzate.

Films removed from Petri dishes were examined.
2.2. Determination of film color characteristics and microstructure

The finished films were assessed for thickness using a micrometer, opacity using a KFK-2MP photoelectrocolorimeter (Zagorsk Optical-Mechanical Plant, Russia) at a wavelength of 600 nm, and color characteristics (lightness (L*), redness/greenness (a*) and yellowness/bluishness (b*)) using an NR60CP colorimeter (Shenzhen Threenh Technology Co, LTD, China).

To determine opacity, formula (1) was used:

\[
\text{Opacity} = \frac{\text{absorbance at 600 nm}}{\text{film thickness (mm)}}
\]  

(1)

A white standard plate was used to calibrate the device, and also as a background when measuring the color characteristics of films (L* = 96.77, a* = 0.11, b* = -0.71). The total color difference (ΔE) and Chroma were calculated using formulas (2) and (3) [7].

\[
\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2}
\]  

(2)

\[
\text{Chroma} = \sqrt{a^2 + b^2}
\]  

(3)

where, L*, a*, b* were the standard color parameter values of the white plate, and L, a, b were the color parameter values of the films.

Appearance was evaluated visually in daylight. To study the morphological characteristics, scanning electron microscopy (SEM) was used to visualize the surface topography and cross section of the films. The films were coated with a thin layer of gold in a high-vacuum coating system (MSP-30T, Showa Shinku Devices Inc., Sagamihara, Japan), and were microscopes on a high-resolution transmission electron microscope with scanning transmission electron microscopy (SEM) function (Jeol JEM-2100, Tokyo, Japan) at magnifications of ×500.

Each sample was analyzed in triplicate.

3 Results and Discussions

Transparency and color films are important characteristics that allow the consumer to visually assess the quality of the product [8]. Also, the optical properties of films affect the penetration of ultraviolet rays into the product and the safety of individual food nutrients.

The appearance and microstructure of the films are presented in Figure 1. Films containing protein hydrolyzate did not visually differ from control samples. To study the internal structure of the films, cross sections were examined using SEM.

As shown in Figure 1, the cross-sections of the hydrolyzate-doped films had smoother and more uniform surfaces compared to the control samples, demonstrating the excellent compatibility between the used structurants and the protein hydrolyzate. Granular particles ranging in size from 1 to 10 micrometers, observed in cross sections of control film samples, lead to uneven structure.

The results of determining color characteristics and opacity are shown in Table 1.
Table 1. Color characteristics and transparency of films

<table>
<thead>
<tr>
<th>Sample</th>
<th>L (lightness)</th>
<th>a* (redness/greenness)</th>
<th>b* (yellowness/bluishness)</th>
<th>ΔE</th>
<th>Opacity, D_{600}/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-C</td>
<td>96.95±0.20</td>
<td>-0.08±0.00</td>
<td>0.29±0.01</td>
<td>1.03</td>
<td>0.69±0.03</td>
</tr>
<tr>
<td>Al-PH</td>
<td>98.34±0.22</td>
<td>-0.63±0.01</td>
<td>0.22±0.01</td>
<td>1.97</td>
<td>0.98±0.04</td>
</tr>
<tr>
<td>P-C</td>
<td>95.00±0.18</td>
<td>0.11±0.01</td>
<td>0.84±0.00</td>
<td>2.35</td>
<td>2.12±0.04</td>
</tr>
<tr>
<td>P-PH</td>
<td>94.71±0.09</td>
<td>0.11±0.01</td>
<td>1.33±0.02</td>
<td>2.90</td>
<td>3.87±0.05</td>
</tr>
<tr>
<td>Ag-C</td>
<td>94.72±0.12</td>
<td>0.16±0.01</td>
<td>0.99±0.01</td>
<td>2.66</td>
<td>0.26±0.02</td>
</tr>
<tr>
<td>Ag-PH</td>
<td>95.46±0.22</td>
<td>0.24±0.02</td>
<td>0.77±0.01</td>
<td>1.98</td>
<td>0.29±0.02</td>
</tr>
</tbody>
</table>

Fig. 1. Appearance and microstructure of films
The results show that the opacity of the film did not differ significantly between the control and experimental agar-based samples. In other film samples with the addition of protein hydrolyzate, a deterioration in transparency was observed.

The brightness index L turned out to be higher for samples of films on alginate.

The green color (negative values), expressed by the a* coordinate, decreased from 0.63 for the alginate-based film sample with the addition of protein hydrolyzate to 0.08 for the control sample. For samples of films on pectin, the values of this indicator turned out to be the same, and samples on agar showed the opposite trend of this indicator - with the addition of protein hydrolyzate, the greenness increased. The yellow color is expressed by positive values of the b* coordinate for all film samples. At the same time, significant differences were established between the control and prototypes on all bases. Films based on pectin with the addition of protein hydrolyzate had the greatest yellowness. Similar results were obtained when studying the color characteristics of agar films with the addition of fish protein hydrolyzate. The authors noted an increase in the yellowness of the film when adding protein hydrolyzate [9].

As the values of the total color difference (ΔE) show, the addition of protein hydrolyzate to alginate and pectin increased this indicator for these films, as well as the color intensity – Chroma (Fig. 2).

![Films Chroma](image)

The literature notes that the addition of protein hydrolyzate and an increase in its amount in the composition of the alginate film led to darkening, the appearance of more reddish and yellowish shades [10].

4 Conclusion

The results of determining color characteristics showed different effects of introducing protein hydrolyzate into alginate, agar and pectin film bases. The addition of protein hydrolyzate affects the transparency of the films, as well as their color characteristics and color intensity, however, the nature of these changes directly depends on the structure-forming biopolymer matrix used.

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