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Physical properties investigation of polylactic acid marker film with anthocyanins extracted from red cabbage and beetroot

Alizadeh, L. [1], **Jahanbakhshian, N.** [2, 3*], **Azizi, M. H.** [4], **Mousavi Nodoshan, R.** [5], **Ehsani, M.** [6]

1. PhD Student, Department of Food Science and Technology, Islamic Azad University, North Tehran Branch, Tehran, Iran.
2. Assistant Professor, Department of Food Science and Technology, Islamic Azad University, North Tehran Branch, Tehran, Iran.
3. Assistant Professor, Department of Food Science and Technology, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
4. Professor, Department of Food Science and Technology, Tarbiat Modares University, Tehran, Iran
5. Associate Professor, Department of Food Science and Technology, Islamic Azad University, North Tehran Branch, Tehran, Iran.
6 Professor, Iran Polymer and Petrochemical Research Institute, Tehran, Iran.

ABSTRACT

In the present study, the properties of film markers of polylactic acid and anthocyanins extracted from red cabbage and beetroot were investigated. There were four samples of: 1 (polylactic acid without anthocyanins), 2 (polylactic acid containing red cabbage anthocyanins), 3 (polylactic acid containing beet anthocyanin) and 4 (polylactic acid containing beet anthocyanin and red cabbage). The samples were tested for thickness, solubility, mechanical properties (tensile strength, elongation at break, Young's modulus) and changes in color indices were also analyzed. The results showed that there was no statistically significant difference in the thickness of the film samples ($p > 0.05$). The highest solubility belonged to the sample 1 and the lowest solubility belonged to sample 4. The results of mechanical tests showed that the highest increase in elongation at breakbelonged to samples 3 and 4 and the lowest increase in elongation at breakbelonged to sample 1 ($p \leq 0.05$). The lowest tensile strength and Young's modulus belonged to sample 1 ($p \leq 0.05$). In all the film samples except sample 1, by increasing pH (up to pH = 14), L * significantly increased ($p \leq 0.05$). In addition, in all pH ranges, sample 1 had the highest L * and sample 3 had the lowest ($p \leq 0.05$). a * in sample 3, at pH = 1-6, had an increasing trend and then decreased to pH = 14 ($p \leq 0.05$). In samples 2 and 4 at pH = 1-13, a decreasing trend was observed and then up to pH = 14 an increasing trend was observed ($p \leq 0.05$). In sample 3, up to pH = 6, an increasing trend and then up to pH = 14, a decreasing trend was observed ($p \leq 0.05$). In the b * of sample 2, first at pH = 1-12, a decreasing trend was observed and then up to pH = 14, an increasing trend was observed ($p \leq 0.05$). The b * of sample 3 at pH = 1-11, and in sample 4 at pH = 1-12, showed a decreasing trend and an increasing trend for both samples up to pH = 14 ($p \leq 0.05$).Sample 4 was introduced as the best treatment due to suitable physical properties and color changes at different pH.

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*Corresponding Author E-Mail:
jahanbakhshian.n@gmail.com

1. Introduction

In recent years, smart packaging[1], due to its ability to trace the safety of food, it has been of great interest. By understanding and sensing some of the features related to the safety of packaged foods and responding to environmental conditions, smart packaging can inform or warn consumers about the status of these features. They examine the packaged food and provide information about the quality of the packaged food while storing, distributing and selling [1]. Intelligent packaging (IP) can control the quality changes of food during storage and transmit information about food quality effectively. The main part of IP is sensors or indicators to estimate and communicate with food quality. For consumers, IP can provide a safer, easier, more interactive and enjoyable experience. For suppliers and sellers, it can reduce costs, achieve more effective supervision in procurement, improve product value and increase profits [2]. Smart packaging technologies are usually divided into three categories: indicators, sensors and RFID (radio frequency identification) tags. By reviewing and summarizing many researches, it was found that the smart packaging used to determine the freshness of food products can be divided into three categories: Indicators[2]Freshness, sensor[3]Newness and tag of radio frequency identification (RFID)[4]). Several types of smart packaging such as O detectors$_2$ و WHAT$_2$, indicator[5]pH, humidity, temperature and time sensors, and pathogenic bacteria biosensors[6] have been introduced so far [3, 4]. The function of smart packaging can be by changing color based on temperature-time, which can be used to indicate the freshness of food or the presence of gases. In general, food spoilage is closely related to pH changes. Therefore, the visual display can show the information directly by changing the visible color. Visual indicator films of two important components of solid support[7] And colors that are sensitive to pH changes are formed. The use of indicators in the form of a smart packaging is one of the things that can be investigated in the meat and aquatic industries [5]. Smart packaging requires the use of pH sensors and indicators. This type of packaging actually communicates with the consumer and informs him whether the product is healthy or unhealthy. When fish spoils, many volatile nitrogen compounds such as biogenic amines, ammonia, trimethylamine and dimethylamine are released, which causes a change in the pH level, that is, the pH of the fish becomes alkaline, which is shown by the reagents. it is possible. Seafood is highly perishable and has a short shelf life, so with the use of appropriate indicators, the process of spoilage can be predicted. In recent years, the use of polylactic acid (PLA) films has become very common due to their transparency. The raw materials of PLA production are agricultural waste such as sugar beet pulp, sugarcane, corn and cheese juice [6]. Polylactic acid is transparent like PET and has glass properties and high clarity [7]. Anthocyanins can be used to check spoilage through color change. Natural anthocyanins are safe, non-toxic and water-soluble pigments that are sensitive to pH changes [8]. Some natural anthocyanins, such as anthocyanins obtained from berries, black beans, and blueberries, have been used to monitor the freshness of food products [9]. Anthocyanins are converted into different chemical forms at different pH. In the systems outside the living organism, anthocyanins can be in different forms due to different pH. In acidic pH ($pH < 2$), anthocyanins are in the form of flavylium cations (in red color soluble in water) and in an alkaline environment ($pH = 4\text{-}7$) they turn into a bluish pink color. The dehydration process of anthocyanins leads to the instability of the structure of anthocyanins and loss of color at this pH [10]. Zhang et al. (2019) applied anthocyanins extracted from rose to the starch-chitosan indicator film and found that the visual color of the indicator film changed from red to yellow when basic volatile nitrogen compounds were formed in pork meat. This study showed that visual indicator films based on anthocyanin are a very good choice of gas sensors to check the freshness of pork [11]. Li et al. (2019) in the investigation of a new indicator film of

1. Intelligent packaging
2. Indicators
3. Sensors
4. Radio Frequency Identification
5. pH indicators
6. pathogenic bacteria biosensors
7. Solid support

carboxymethyl cellulose (CMC)/starch (S) and purple sweet potato anthocyanins.[8] (PSPA) stated that according to the Fourier Transform Infrared Spectroscopy (FT-IR) analysis, new interactions have been created between the components of the film and the results of Scanning Electron Microscope (SEM) and X-Ray Diffraction (XRD) showed that PSPA is well in CMC and dispersed starch based films. The results of differential scanning calorimetry (DSC) analysis showed that the melting temperature of the new film increased. Adding PSPA to the film led to an increase in tensile strength, but a decrease in breaking length and a decrease in moisture content were observed. Color change analysis showed that when exposed to changes in pH and ammonia, the color of the film changed from red to blue and green [12]. Vo and colleagues (2019) with the aim of producing smart pH indicator films from chitosan/poly(vinyl alcohol[9])/ used anthocyanin extracted from red cabbage. In this way, pH indicator films are successfully made from hydrogels[10] with a combination of 1% poly (vinyl alcohol) (PVA) and 1% chitosan (CS) with anthocyanin (ATH) and sodium tripolyphosphate ([11]STPP) were prepared. In particular, ATH extracted from red cabbage was used as a pH indicator, while STPP was used as a cross-linking agent to provide better mechanical properties. The characteristics of the films such as FT-IR spectroscopy confirmed the presence of ATH in the films. In addition, tensile strength[12], tensile length at failure[13] and inflation indices[14] The films were measured. In general, these properties of pH indicator films were deeply influenced by PVA/CS compounds and STPP dose applied in hydrogels. For example, the tensile strength of the film prepared from pure PVA hydrogel increased from 43.27 MPa to 29.89 MPa, when 35% of the PVA hydrogel was replaced with CS [13]. Chen et al. (2020) used extracts containing curcumin and pH-sensitive anthocyanins to prepare a sensitive indicator film to detect the freshness of fish meat. The proposed indicator film was based on starch, polyvinyl alcohol and glycerol, and the desired extract was added to the polymer matrix. These indicator films produced three different colors (yellow, purple, and brown), which indicated the freshness, semi-freshness, and spoilage of the fish [14]. Alizadeh-Sani et al. / Methyl cellulose content of saffron anthocyanin stated that the main groups that played a role in the molecular interactions between the different components in the films. There were hydroxyl groups (anthocyanin, methyl cellulose and chitosan nanofiber) and amino groups (chitosan nanofiber). In addition, ATR-FTIR analysis showed that the O-H groups on saffron anthocyanin formed hydrogen bonds with O-H and N-H on the chitosan/methyl cellulose nanofiber matrix, and the films that were made of only methyl cellulose had smooth and uniform surfaces. The films containing chitosan and methyl cellulose nanofibers had a harder surface morphology, which was attributed to the protrusion caused by the nanofibers and their surface. And the integration of anthocyanins led to a slightly smoother surface morphology, which was attributed to their ability to interact with other compounds in the structure. Pure methyl cellulose films were relatively strong, rigid and flexible. The color of the solutions changed from red to yellow with increasing pH from 1 to 14, and a number of different colors were created at medium pH values: red/pink (pH 1-4). Purple/grey (pH 5-6), green (pH 7-9) and yellow-green/yellow (pH 10-14). These color changes were attributed to structural changes of anthocyanin molecules caused by pH [15]. In this research, the prediction of salmon spoilage was investigated using polylactic acid indicator film and anthocyanins extracted from red cabbage and beetroot. The review of sources shows that so far there has been research on the use of polylactic acid indicator films. With these two types of anthocyanins, it has not been done to determine the spoilage of meat products, considering the abundance of red cabbage and beetroot in Iran, and considering the distinct feature of PLA film (transparency), which clearly shows the color

8. Purple sweet potato anthocyanins
9. Poly(vinyl alcohol
10. Hydrogels
11. Sodiumtripolyphosphate
12. Tensile strength
13. Elongation-at-break
14. Swelling indices

changes, the importance of the present research is clear. be made

2- Materials and methods

2-1- Extraction of anthocyanin pigment by solvent and under ultrasound from red cabbage and beetroot

In order to extract anthocyanin, 10 grams of red cabbage and beetroot samples were placed in 40 ml of solvent (ratio 4:1) in a laboratory ultrasonic bath (model vCLEAN1 - L20). The solvent used included methanol and hydrochloric acid in a ratio of (99:1). . The sonication process was set at 2, 5 and 10 minutes and the intensity of ultrasound was 200, 300 and 400 ultrasound. The samples were also prepared under the same conditions but without the use of ultrasonic waves. Anthocyanin absorbance of the extract obtained at pH = 1 and pH = 4.5 was measured at a wavelength of 510 nm using a spectrophotometer and the concentration of anthocyanins in the extract was calculated by the differential pH method. In the last step, the extracted extract was concentrated by a rotary evaporator device at a temperature of 50 °C for 2 hours until Brix 60 [16].

2-2- Production of polylactic acid films

Solvent casting method and the method presented by Rhim et al. (2006) were used to prepare the polylactic acid film. 5 grams of polylactic acid granules were added to 100 milliliters of chloroform and the resulting mixture was stirred for 8 hours on a magnetic stirrer at room temperature. Then, anthocyanin was added to the amount of 1% of the dry matter of the films. It should be noted that in the present research, four films were prepared so that a control film (lacking anthocyanin) was considered. In the second type, it was prepared only from red cabbage anthocyanin, in the third type from beetroot anthocyanin, and in the fourth film from a 50-50 ratio of red cabbage and beetroot anthocyanins). The resulting mixture was placed on a magnetic stirrer for another 20 minutes. At the end, it was homogenized with a homogenizer (Korea, 15D Wise) for 2 minutes at 12,000 rpm. Different levels of anthocyanin were added to the final solution and homogenized at 8000 rpm for 10 minutes and degassing was done by sonicator for 30 minutes at room temperature. Then the solution was poured into glass molds and kept for 4 hours at room temperature under a chemical hood. After the evaporation of the solvent, the prepared films were separated from the molds and placed at room temperature for 24 hours to completely remove the residual solvent that may have a plasticizing role. After preparing polylactic acid films containing anthocyanin, discs with a diameter of 13 mm were punched [17].

2-3- Tests of samples of pH indicator films

2-3-1-Thickness

The thickness of the prepared films was measured using a micrometer with an accuracy of 0.001. At least 3 different points were measured in each film and the average of the numbers obtained in the required tests was used [18].

2-3-2- Solubility

Pieces of the film (600 mg) cut in a desiccator with (P_2O_5 (0% RH is heated for 24 hours at a temperature of 40 degrees Celsius. Then 100 cc of deionized water is placed in the beaker, these samples are stirred with constant shaking for 24 hours at room temperature. Then the mixture of film and water is placed on a paper The filter that has already reached a constant weight and has been accurately weighed is filtered. The filter paper is placed with the sample at a temperature of 40 degrees Celsius until the constant weight is reached. The solubility percentage of the films in water is calculated from the following equation [19].

= Solubility percentage

Final dried weight of the film - initial dried weight of the film/Initial dried weight of the film ×100

2-3-3-Mechanical properties

The mechanical tests of the films were performed based on the modified ASTM D0882-02 method. The films were cut into pieces of 1 x 7 cm and conditioned under the conditions of relative humidity of 50% and temperature of 25 degrees Celsius. Before performing the mechanical test, the thickness of the films was

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determined at 5 measurement points and their average thickness. In the Instran device, the distance between the two jaws was 50 mm, the upper jaw movement speed was 50 mm/min and the lower jaw was constant. The amount of elasticity (percentage) by dividing the amount of tension at the moment of breaking the film sample by the initial length of the film, the tensile strength (megapascals) by dividing the maximum force on the initial surface and the elastic modulus (megapascals), the slope of the first part of the curve, whose slope is constant were obtained [20].

$$TS=\frac{Maximum\ load}{Original.minimum.cross.sectional.area}=\frac{\Delta L}{L0}\times 100$$

$$\%\ E=\frac{Extension\ at\ moment\ of\ rupture}{initial\ gage\ lengh}=\frac{\Delta L}{L0}\times 100$$

In relation 4, ΔL is the amount of stretching until the moment of rupture in millimeters; L is the initial length of the film or the initial distance between the two jaws in millimeters, and E is dimensionless.

2-3-4- Colorimetry (b* a* L* values)

In the present research, first, buffer solutions with different pH were prepared and poured into a 15 ml falcon, and then film samples with dimensions of 1x1 cm were placed in it, and after 2 hours, the films were taken out and their color was evaluated. The test to determine the color of film samples by a colorimeter device (Hunter Lab[15]) according to AOCC method No. 22-01/14, which determined the color parameters of the samples by specifying the color indices a*, b* and L* [21].

2-4- Statistical analysis

The experimental data are compared with one-way ANOVA. Statistically significant differences between mean values were determined using Duncan's multi-range follow-up test. Statistical tests of the obtained results were performed using SPSS version 26 software. The significance level is 0.05$p\leq$ was considered for all data comparisons and Excel software was used to draw graphs.

15. Hunter-Lab

3- Results and discussion

3-1- Extraction results of anthocyanin pigments extracted from red cabbage and beetroot

The results of comparing the average anthocyanin pigment of the samples are presented in Table 1. According to the average comparison results, the highest amount of anthocyanin in red cabbage was observed at 200% sound intensity and 5 and 10 minutes time (0.05).*p*Also, the highest amount of anthocyanin in beetroot was related to sound intensity of 300% and time of 5 minutes (0.05$p\leq$). The main mechanism of ultrasound extraction is related to the phenomenon of cavitation, during which very small bubbles are formed in the liquid, quickly grow to a critical size and explode. Therefore, the use of these waves in the extraction of various compounds from plant tissues increases the efficiency and speed of the extraction process and reduces the solvent consumption [22]. Ultrasonic waves have mechanical effects that increase the permeability of the solvent in cellular tissues and in Finally, they lead to better and faster mass transfer. Living cells are destroyed under the influence of ultrasound waves and release their contents better and easier. Among the advantages of this method, we can mention increasing the efficiency of the process and increasing the speed of operation [23]. The use of ultrasound in the extraction process increases the efficiency and speed of the extraction process and reduces the solvent consumption [24]. Shukla et al. (2016) used indicator sensors based on anthocyanin extracted from roses and red cabbage and filter paper as a stationary carrier for smart packaging and stated that the study of the extracts by UV-Vis spectrophotometer, the maximum absorption of 530 nm and it was attributed to anthocyanin present in two extracts [25]. Rouhani et al (2015) used three times (5, 10 and 15 minutes) and three sound intensities (20, 60 and 100%) in the extraction of anthocyanin and antioxidant compounds of saffron flower stamens with the help of ultrasound waves and found that with increasing time and The sound intensity increased from 5 to

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10 minutes, but the amount of total polyphenol increased, but at 15 minutes, this trend was fixed and decreased in some cases. The optimal extraction conditions were 10 minutes and 100% sound intensity [26]. Simona Oancea et al. (2018) with the aim of extracting antioxidant components from red onion skin lesions with the help of ultrasound waves, stated that the highest amount of flavonoid, anthocyanin and phenolic compounds were extracted in the shortest time by these waves with a ratio of 30 to 1 solvent to solid substance. And the highest amount of antioxidants was obtained in 20 minutes in the vicinity of ultrasound waves [27]. Pedram Nia et al. (2018) studied the extraction of barberry anthocyanin in the presence of ultrasound waves at three different temperatures of 30, 40, 50 and three times of 10, 20, and 30 minutes with two solvents, hydrochloric acid and ethanol. In this study, it was found that the highest amount of anthocyanin was in the extract and pulp that was extracted using ultrasonic waves at a temperature of 50 degrees and a time of 20 minutes, and its amount was reported as 260.171 mg per 100 ml of solution [28] [.

Table 1 Results of the amount of anthocyanin pigments extracted from red cabbage and beetroot under Ultrasound-assisted extraction

Source of anthocyanins		Intensity	Time
beetroot	Red cabbage	(%)	(min)
579.7853 [d]	268.852 [b]	200	2
584.461[c]	282.8791 [a]	200	5
565.7582 [It is]	285.217 [a]	200	10
589.1367 [b]	261.8385 [d]	300	2
607.8394 [a]	271.1899 [c]	300	5
575.1096 [d]	254.825 [It is]	300	10
551.7312 [f]	243.1358 [f]	400	2
542.3798 [g]	245.4736 [f]	400	5
537.7041 [g]	236.1222 [g]	400	10

Different lower case letters indicate a significant difference in the column ($p<0.05$).

2-3- Test results of film samples

3-2-1- Thickness

The results of the present research (Table 2) showed that there was no significant statistical difference in the thickness of the film samples (0.05).*p*>). Chen et al. (2012) during the production of smart films from composites of nanocrystal chitosan/chitin oxide (CS/OCN) and red cabbage anthocyanins (RCA) to monitor the freshness of fish and shrimp, stated that the thickness of each film is influenced by the content of RCAs. It was not found that it was given to small added amounts and favorable compatibility between CS/OCN and RCA composites [29].

3-2-2- Solubility

The results of the present research (Table 2) showed that the highest solubility belonged to the control sample and the lowest amount of solubility belonged to sample 4. Solubility is one of the important factors of biodegradable films that affect the film's resistance to water, especially in humid environments [30]. Wang et al. (2012) in investigating the effect of anthocyanin-rich red raspberry extract (ARRE) on the characteristics of edible soy protein isolate (SPI) films, stated that the addition of anthocyanin extract led to a decrease in the solubility of the samples. anthocyanins, the solubility of ruby reduction film samples [31]. Merz et al. (2020) in the study of a color indicator film based on chitosan, polyvinyl alcohol and anthocyanin extracted from jumbo fruit *(Syzygium Cumini)* In order to monitor the freshness of shrimp, they stated that adding levels of 0, 1, and 3% of anthocyanin to the films did not create a statistically significant difference in the solubility of the film samples, which may be the reason for the difference with the findings of the current research. attributed anthocyanin [32].

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Table 2 Results of solubility and thickness of film samples

Thickness (mm)	Solubility (%)	
0.29±0.01[a]	13.70±.018[a]	Cod (1)
0.30±0.01[a]	12.88±0.12[b]	Cod (2)
0.29±0.01[a]	12.56±0.23[b]	Cod (3)
0.30±0.01[a]	11.30±0.35[c]	Cod (4)

Different lowercase letters indicate a significant difference in the column (p <0.05).
Cod (1):Polylactic acid film without anthocyanin, Cod (2):Polylactic acid film with anthocyanins extracted from red cabbage, Cod (3): Polylactic acid film with anthocyanins extracted from beetroot, Cod (4): Polylactic acid film with anthocyanins extracted from red cabbage and beetroot

3-2-3-Mechanical properties

3-2-3-1-Increasing length at break (flexibility)

The results of the present research (Table 3) showed that the highest elongation at break belongs to samples 3 (polylactic acid film containing beetroot anthocyanins) and 4 (polylactic acid film containing red cabbage and beetroot anthocyanins) and the lowest length increase in The failure belonged to sample 1 (polylactic acid film without anthocyanin) (0.05*p*≤). In general, the mechanical properties are under the influence of several factors, including the interactions between film compounds, temperature, physical and chemical conditions [33]. The maximum tension that the film can withstand until it breaks (TS) in the edible film depends on the chemical structure of the molecules or the connection of the polymer chains in the sheet matrix of the film [34]. In general, the films must be resistant to the pressures that are applied to it during use and transportation in order to maintain its strength as well as its barrier properties. The mechanical properties of films are one of the important elements for their selection and use for packaging purposes and are as important as their inhibition properties. Usually, a film is desirable from a mechanical point of view, which at the same time has high mechanical resistance, high stretchability and flexibility, and is not brittle or brittle [35]. Alizadeh-Sani et al. (2021) in the study of the indicator composite films consisting of chitosan/methylcellulose nanofiber containing saffron anthocyanin stated that the pure methylcellulose films were relatively strong, rigid and flexible. The combination of chitosan nanofibers (3%) significantly increased the strength and stiffness of the films and reduced their flexibility. On the contrary, the composition of saffron anthocyanin in composite films slightly reduced their strength and stiffness and increased their flexibility, which shows that chitosan nanofibers and anthocyanins have influenced the structure or interactions of biopolymers and particles in composite films. [15]. Anthocyanins may act as softeners that increase the mobility of biopolymer chains and thus increase the flexibility of the film [36, 37]. Researchers have reported that black plum skin anthocyanins increase the mechanical resistance and flexibility of chitosan films [11]. While other researchers have reported that sweet potato anthocyanins reduce the strength and increase the flexibility of chitosan films (Yong et al., 2019). As a result, it seems that the effect of anthocyanins depends on the system. Li and colleagues (2019) in the production of the indicator film consisting of carboxymethyl cellulose (CMC)/starch (S) and purple sweet potato anthocyanins admitted that the addition of anthocyanins to the film led to an increase in tensile strength, but a decrease in breaking length and A decrease in humidity was observed.

3-2-3-2-tensile resistance

The results of the current research (Table 3) showed that the lowest tensile strength belonged to sample 1 (polylactic acid film without anthocyanin) (0.05).*p*≤). In general, elongation or elongation to breaking point (EB) is defined as the percentage increase in the length of the sample at the time of rupture (Longares*et al.*, 2004) and in other words, the maximum length at which the film breaks is the percentage of the increase in the length of the film. High elasticity of the film is always a desirable characteristic, which is characterized by a high percentage of elongation at break**..** Vo et al. (2019) with the aim of producing pH indicator smart films from chitosan/poly (vinyl alcohol)/anthocyanin

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extracted from red cabbage, stated that the properties of the films were deeply affected by the PVA/CS compounds and the STPP dose applied in the hydrogels. . For example, the tensile strength of 43.27 MPa on the film prepared from pure PVA hydrogel reached 29.89 MPa, when 35% of PVA hydrogel was replaced with CS.

3-2-3-3-Young's modulus

The results of the present research (Table 3) showed that the lowest Young's modulus belonged to sample 1 (polylactic acid film without anthocyanin) (0.05).*p*≤). Coefficient of elasticity shows the hardness of the film (*et al*(Lazarus, 1976). Young's modulus or modulus of elasticity is said to be the ratio of stress to strain of linear solids below the yield strength, in which case Hooke's law is true and the elastic modulus is constant. The exponent is the amount of force required to change the shape of an elastic body to a certain extent (Longares*et al*., 2004).

Table 3 Results of mechanical test of film samples

Young's modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)	
0.47±0.03[b]	0.008±0.000[b]	187.08±5.63[c]	Cod (1)
0.59±0.04[a]	0.018±0.000 [a]	315.54±21.15 [b]	Cod (2)
0.51±0.04[ab]	0.019±0.000 [a]	368.54±14.28 [a]	Cod (3)
0.54±0.03[ab]	0.019±0.000 [a]	354.29±33.21 [ab]	Cod (4)

Different lower case letters indicate a significant difference in the column (p<0.05).

Cod (1):Polylactic acid film without anthocyanin, Cod (2):Polylactic acid film with anthocyanins extracted from red cabbage, Cod (3): Polylactic acid film with anthocyanins extracted from beetroot, Cod (4): Polylactic acid film with anthocyanins extracted from red cabbage and beetroot

3-3- Evaluating the results of changes in color components b*, a*, L* with pH changes

3-3-1- Color component L*

The results of the present research (Table 4) showed that in all film samples except sample 1 (polylactic acid film without anthocyanin), with increasing pH (up to pH=14), the color component L* of the samples increased significantly (0.05).*p*≤) so that the highest amount of color component L* was observed at pH=14 and the lowest amount was observed at pH=1 (0.05*p*≤). Also, in all the investigated pHs, sample 1 (polylactic acid film without anthocyanin) had the highest color component L* and sample 3 (polylactic acid film containing beetroot anthocyanin) had the lowest color component L* compared to other samples (05 / 0*p*≤). Color index L* measures the degree of darkness and brightness (light-dark, 0-100). (Pino & Gonzalez, 2002). Pereira et al. (2015) in the study of anthocyanin (ATH) extracted from red cabbage stated that anthocyanin at a concentration of 86.67 mg/liter, with buffer solutions from pH 1 to pH 13 The color change showed clearly. The advantage of using anthocyanins as a pH indicator is due to their obvious color change at different pH levels. For example, their color is red or pink in acid solutions and blue or green in alkaline solutions. As a result, ATH has been suitable as a pH indicator in films consisting of PVA, CS and STPP [9]. Alizadeh-Sani et al. (2021) in the study of indicator composite films consisting of nanofiber chitosan/methyl cellulose containing saffron anthocyanin stated that the color of the solutions changed from red to yellow with increasing pH from 1 to 14 and a number of different colors were created at medium pH values: Red/pink (pH 1-4). Purple/gray (pH 6-5), green (pH 7-9) and yellow-green/yellow (pH 14-10) [15]. These color changes were attributed to structural changes of anthocyanin molecules caused by pH: flavylium cation[16](pH <3). Carbinol pseudobase[17](pH 4-5) anhydrous quinonoidal base[18](pH 6-8) and chalcone[19](pH > 10) [11]. The intensity and wavelength of the maximum absorption peak of anthocyanin

16. Flavylium cation
17. Carbinol pseudo-base
18. Quinonoidal anhydro-base
19. Chalcone

solutions depends on pH. In very acidic conditions (pH < 4), a strong absorption peak was observed at about 520 nm, and its height decreased with increasing pH. Then a new peak was formed at about 550 nm, which increased in intensity and position as the pH increased from 5 to 9, which is a characteristic of anthocyanins [41]. The intensity of the peak decreased when the pH increased from 10 to 14. The change in absorption spectrum and color of saffron anthocyanin solutions can be attributed to changes in existing molecular species. Similar findings for anthocyanins isolated from black plum skin [11]. and blueberries have been reported [41]. Li et al. (2019) in the production of indicator film composed of carboxymethyl cellulose (CMC)/starch (S) and violet sweet potato anthocyanins admitted that according to the results of color change analysis, when the film is exposed to pH changes and Ammonia, the color of the film changed from red to blue and green. Also, SCA film was used as labels to monitor the freshness of fish meat kept at 20 °C. The results showed that when the fish was spoiled, the color of the film changed from red to blue, and the results were consistent with the amount of TVB-N, which was higher than the determined limit (20 mg/100 g) [12]. Kuswandi et al. (2012) from a polyaniline-based indicator film produced using a polystyrene substrate to detect fish spoilage stated that there is a good relationship between the response of the indicator film and microbial growth patterns in fish samples, especially the change in the microbial population (number acceptable total (TVC) and Pseudomonas) and with the increase of microbial population, the color of the film gradually changed from green to blue, and they generally acknowledged that the color changes, in terms of overall color changes, have a good relationship with the TVBN level of the fish] 42 [. Chun et al. (2014) in the production of an indicator film to check the freshness of fish in real time by the rotation of bromocresol green color on the filter paper stated that the color of the indicator film gradually changes in response to the increase of volatile nitrogen compounds, which leads to an increase in the pH of the fish sample. changed from yellow to green [43].

Table 4 Changes in the L* of films with pH changes

Cod (4)	Cod (3)	Cod (2)	Cod (1)	
31.74±0.25^{iC}	28.78±0.64^{hD}	36.52±0.34^{jB}	76.71±0.19^{aA}	pH=1
32.29±0.63^{iC}	31.59±1.00^{gD}	37.10±0.33^{iB}	76.78±0.45^{aA}	pH=2
36.79±0.53^{gC}	31.27±0.26^{gD}	46.09±0.22^{gB}	76.76±0.46^{aA}	pH=3
38.56±0.46^{fC}	33.89±0.32^{fD}	46.76±0.33^{fB}	76.77±0.25^{aA}	pH=4
39.53±0.36^{eC}	35.093±0.13^{deD}	47.31±0.53^{eB}	76.27±0.09^{abA}	pH=5
43.13±0.24^{cC}	37.78±0.35^{cD}	51.28±0.04^{cB}	76.13±0.16^{abA}	pH=6
41.59±0.27^{dC}	35.89±0.51^{dD}	50.65±0.43^{dB}	75.99±0.58^{not}	pH=7
41.08±0.23^{dC}	37.83±0.29^{cD}	47.50±0.33^{eB}	75.72±0.30^{bcA}	pH=8
40.01±0.12^{eC}	31.55±0.44^{gD}	51.57±0.14^{cB}	75.67±0.39^{bcA}	pH=9
35.69±0.49^{hC}	38.22±0.54^{cD}	36.72±0.15^{ijB}	75.15±0.41^{cdA}	pH=10
41.12±0.72^{dD}	47.98±0.13^{bC}	37.88±0.22^{hB}	75.28±0.38^{cdA}	pH=11
38.81±0.20^{fC}	34.29±0.41^{efD}	45.78±0.16^{gB}	74.64±0.63^{deA}	pH=12
54.33±0.22^{bC}	52.15±0.42^{aD}	60.43±0.29^{bB}	74.06±0.35$^{of\ A}$	pH=13
55.56±0.11^{BC}	52.59±0.51^{aD}	61.48±0.28^{aB}	73.99±0.35$^{of\ A}$	pH=14

Different lowercase letters indicate a significant difference in the column and different uppercase letters indicate a significant difference in the row (p<0.05).

Cod (1):Polylactic acid film without anthocyanin, Cod (2):Polylactic acid film with anthocyanins extracted from red cabbage, Cod (3): Polylactic acid film with anthocyanins extracted from beetroot, Cod (4): Polylactic acid film with anthocyanins extracted from red cabbage and beetroot

3-3-2-color component a*

The results of the present research (Table 5) showed that in sample 2 (polylactic acid film containing beetroot anthocyanin), the highest amount of a* was observed at pH=1 and the lowest amount was observed at pH=13. In sample 3 (polylactic acid film containing beetroot anthocyanin), the highest amount of a* was observed at pH=6 and the lowest at pH=14 (0.05).*p*≤). In sample 4 (polylactic acid film containing anthocyanins of red cabbage and beetroot), the highest amount of a* belonged to pH 1 and 2, and the lowest amount of a* was observed at pH=13 (0.05).*p*≤). In general, it can be stated that the color component a* in sample 3 (polylactic acid film containing beetroot anthocyanin) had an increasing trend at pH=6-1 and then a decreasing trend until pH=14 (0.05).*p*≤). In samples 2 (polylactic acid film containing red cabbage anthocyanins) and 4 (polylactic acid film containing red cabbage anthocyanins and beetroot) at pH=13-13, a decreasing trend was observed and then an increasing trend was observed until pH=14 (/05). 0*p*≤). In sample 3 (polylactic acid film containing beetroot anthocyanin), an increasing trend was observed until pH=6 and then a decreasing trend was observed until pH=14 (0.05).*p*≤). Color index a* indicates redness in the samples (absolute red-absolute green, 120+-120)]44[. A positive score indicates that the sample is red, while a negative score indicates that the sample is green [45]. Ma et al. (2017) with the combination of anthocyanins extracted from the shell*Amur vine*They produced a new indicator film with a mixture of tara gum and cellulose to investigate the spoilage of fish, in which a detectable color change from pink to green was observed [46]. Zhang et al. (2019) using anthocyanins extracted from rose in the starch-chitosan indicator film, found that when essential volatile nitrogen compounds are formed in pork meat, the visual color of the indicator film changes from red to yellow. This study showed that visual indicator films based on anthocyanin are a very good choice of gas sensors to check the freshness of pork [11]. Li et al. (2019) during the production of a new indicator film from carboxymethyl cellulose (CMC)/starch (S) and purple sweet potato anthocyanins.[20] (PSPA) stated that when exposed to changes in pH and ammonia, the color of the films changed from red to blue and green. In the present research, the redness of the film samples was reduced by increasing the pH [12].

20. Purple sweet potato anthocyanins

Table 5 Changes in the a* of films with pH changes

Cod (4)	Cod (3)	Cod (2)	Cod (1)	
43.36±0.31 eB	40.44±0.25 gC	49.85±0.87 aA	0.20±0.11 aD	pH=1
43.81±0.16 eB	48.46±0.58 fA	44.10±0.23 bB	0.15±0.08 BC	pH=2
30.69±0.82 eB	50.77±0.31of A	12.89±0.34 cC	0.15±0.06 bD	pH=3
26.43±0.37 eB	52.42±0.17 and	3.85±0.38 dC	0.28±0.11 cD	pH=4
27.30±0.46 eB	54.26±0.26 not	2.91±0.06 eC	0.30±0.17 cD	pH=5
26.54±0.35 as B	55.18±0.39 aA	1.18±0.21 fC	0.47±0.24 cD	pH=6
24.57±0.32 cdB	52.89±0.44 that	-1.44±0.34 gD	0.69±0.28 dC	pH=7
20.72±0.19 cdB	48.83±0.62 of A	-4.28±0.16 hD	0.68±0.34 eC	pH=8
14.34±0.64 cB	41.75±0.27gA	-9.12±0.54 iD	0.96±0.17 fC	pH=9
4.89±0.18 bB	27.81±0.62 to A	-15.07±0.57jD	1.34±0.05 gC	pH=10
1.84±0.67 cB	25.71±0.58 and A	-19.11±0.82 lD	1.00±0.29 hC	pH=11
1.68±0.47 bB	24.51±0.29 kA	-18.25±0.73 kD	1.44±0.14 hC	pH=12
-12.19±0.77 BC	1.67±0.49 lB	-22.67±0.50 mD	1.93±0.08 and A	pH=13
-2.08±0.58 aD	-1.34±0.41 mC	0.44±0.21 fB	1.86±0.08 to A	pH=14

Different lower case letters indicate a significant difference in the column and different uppercase letters indicate a significant difference in the row (p<0.05).

Cod (1):Polylactic acid film without anthocyanin, Cod (2):Polylactic acid film with anthocyanins extracted from red cabbage, Cod (3): Polylactic acid film with anthocyanins extracted from beetroot, Cod (4): Polylactic acid film with anthocyanins extracted from red cabbage and beetroot

3-3-3-color component b*

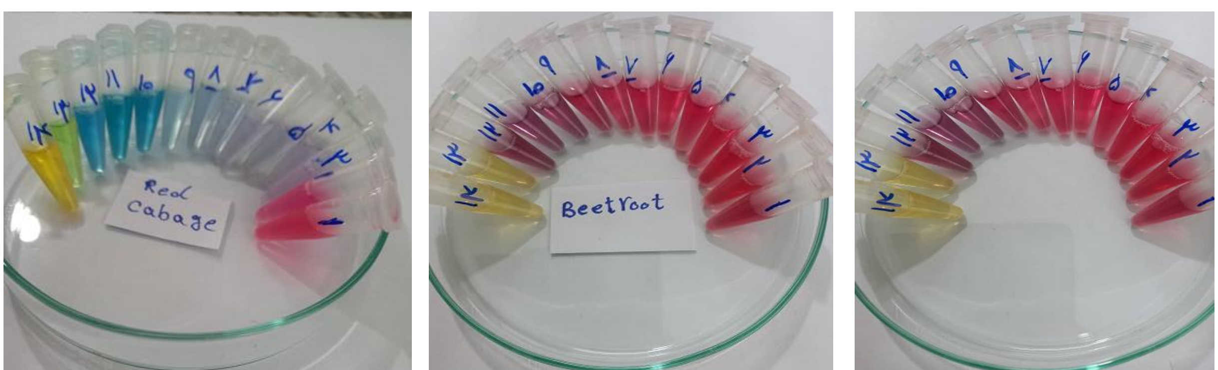
The results of the present research (Table 6) showed that in sample 2 (polylactic acid film containing red cabbage anthocyanin), the highest amount of b* was observed at pH=14 and the lowest at pH=12, and in samples 3 (polylactic acid film lactic acid containing beetroot anthocyanins) and 4 (polylactic acid film containing red cabbage and beetroot anthocyanins), the highest amount of b* was observed at pH=14 and the lowest at pH=11. In general, it can be stated that In the color component b* of sample 2 (polylactic acid film containing red cabbage anthocyanin), first at pH=12-12, a decreasing trend and then an increasing trend was observed until pH=14 (0.05*p*≤). Color component b* of sample 3 (polylactic acid film containing beetroot anthocyanins) at pH=11-11, and in sample 4 (polylactic acid film containing red cabbage and beetroot anthocyanins) at pH=12-12, decreasing trend and Again, it showed an increasing trend for both samples up to pH=14 (0.05*p*≤). The positive value of the color index b* shows the degree of yellowness of the sample and its negative value shows the degree of inclination to the blue color of the sample (the score of the color index b* and the negative degree of the inclination to the blue color of the sample) (absolute yellow - absolute blue) , 120+-120)]11[. Erna et al. (2022) in the investigation of the physicochemical properties of polymer film based on starch containing anthocyanin, stated that the indicator films showed a high sensitivity to pH changes, so that the mentioned films at pH -2.0 6.0 showed bright red color, at pH 7.0-11.0, bluish-gray color and at pH above 11, yellowish-green color [47]. In the present research, according to visual observations (Figure 1), sample 2 (polylactic acid film containing red cabbage anthocyanin) at pH 1-2, bright red and pink colors, at pH 3-6, pale purple color, and at pH 7 -9, gray color, pH 10-12, pale blue color, pale green color at pH=13 and yellow color at pH=14. Also, samples 3 (polylactic acid film containing beetroot anthocyanins) and 4 (polylactic acid film containing red cabbage and beetroot anthocyanins), at pH 1-9, relatively dark to light red colors, at pH 10-12, pale violet, and showed a yellow color at pH 13-14. Chun et al. (2014) prepared a Schanger film to check the freshness of fish in real time by the rotation of bromocresol green color on the filter paper and based on the amount of volatile nitrogen compounds produced in They investigated the color change of mackerel fish fillets during spoilage. The results showed that the color of the indicator film gradually changed from yellow to green in response to the increase in volatile nitrogen compounds, which leads to an increase in the pH of the fish sample [43]. Shukla et al. (2016) used indicator sensors based on anthocyanin extracted from roses and red cabbage and filter paper as immobile carriers for smart packaging. The indicator sensor was based on natural flavonoids (anthocyanin) extracted from red cabbage and rose, which changed its color from red to green with increasing pH [49].

Table 4 Changes in the b* of films with pH changes

Cod (4)	Cod (3)	Cod (2)	Cod (1)	
13.67±0.25 ^{gA}	13.51±0.53 ^{that}	16.23±0.71 ^{cCD}	2.35±0.20 ^{if}	pH=1
11.76±0.19 ^{dB}	22.37±0.32 ^{and}	5.24±0.46 ^{dCD}	2.20±0.11^{fC}	pH=2
9.28±0.34 ^{cB}	23.96±0.36 $^{of A}$	-3.23±0.00 ^{eD}	2.26±0.05 ^{efC}	pH=3
8.11±0.23 ^{dB}	22.97±0.81 ^{fA}	-4.52±0.00 ^{fD}	2.32±0.05 ^{efC}	pH=4
7.52±0.35 ^{cB}	24.00±0.57 ^{fA}	-5.52±0.27 ^{gD}	2.22±0.05 ^{fC}	pH=5
6.60±0.31 ^{eB}	21.06±0.09 ^{gA}	-5.55±0.30 ^{gD}	2.62±0.09 ^{deC}	pH=6
2.28±0.50 ^{fB}	16.48±0.58 ^{ha}	-8.46±0.41 ^{hD}	2.96±0.11 ^{cdC}	pH=7
-1.57±0.43 ^{hC}	9.66±0.59 $^{to A}$	-9.55±0.08 ^{iD}	2.80±0.25 ^{dB}	pH=8
-1.66±0.22 ^{hD}	9.94±0.45 ^{iC}	-10.42±±0.36 $^{and A}$	2.94±0.37 ^{cdB}	pH=9
-13.42±0.42 ^{jC}	-1.48±0.30 ^{kB}	-21.86±0.20 ^{kD}	3.29±0.12^{bcA}	pH=10
-14.25±0.85 ^{kC}	-4.29±0.44 ^{lB}	-22.66±1.01 ^{kD}	3.34±0.34 ^{not}	pH=11
-12.24±0.23 ^{iC}	1.86±0.37 ^{jB}	-24.11±0.57 ^{lD}	3.74±0.29 ^{aA}	pH=12
33.12±0.67 ^{bB}	31.80±0.66 ^{bC}	36.67±0.36 ^{not}	3.61±0.13 ^{USA}	pH=13
45.19±0.54 ^{aB}	33.75±0.22 ^{BC}	59.86±0.42 ^{aA}	3.64±0.19 ^{USA}	pH=14

Different lower case letters indicate a significant difference in the column and different uppercase letters indicate a significant difference in the row (p<0.05).

Cod (1):Polylactic acid film without anthocyanin, Cod (2):Polylactic acid film with anthocyanins extracted from red cabbage, Cod (3): Polylactic acid film with anthocyanins extracted from beetroot, Cod (4): Polylactic acid film with anthocyanins extracted from red cabbage and beetroot



Cod (2) Cod (3) Cod (4)

Fig 1 Visual observations of film color change at different pH.
Cod (2):Polylactic acid film with anthocyanins extracted from red cabbage, Cod (3): Polylactic acid film with anthocyanins extracted from beetroot, Cod (4): Polylactic acid film with anthocyanins extracted from red cabbage and beetroot

4- General conclusion

The results of this research showed that there was no significant statistical difference in the

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thickness of the film samples (0.05).*p*>). The highest solubility belonged to film sample 1 and the lowest amount of solubility belonged to sample 4. The results of mechanical tests showed that the highest elongation at break belonged to samples 3 and 4 and the lowest elongation at fracture belonged to sample 1 (05 / 0*p*≤). Also, the lowest tensile strength and Young's modulus belonged to sample 1 (0.05*p*≤). In all film samples except sample 1, with increasing pH (up to pH=14), the color component L* of the samples increased significantly (0.05*p*≤). Also, in all investigated pHs, sample 1 had the highest color component L* and sample 3 had the lowest color component L* (0.05)*p*≤).The color component a* in sample 3, at pH=6-1, had an increasing trend and then a decreasing trend until pH=14 (0.05*p*≤). In samples 2 and 4 at pH=13-1, a decreasing trend and then an increasing trend was observed until pH=14 (0.05*p*≤). In sample 3, an increasing trend was observed until pH=6 and then a decreasing trend was observed until pH=14 (0.05).*p*≤). In the color component b* of sample 2, first at pH=12-12, a decreasing trend and then an increasing trend was observed until pH=14 (0.05*p*≤). The color component b* of sample 3 at pH=11-1, and in sample 4 at pH=12-1, showed a decreasing trend and again an increasing trend for both samples until pH=14 (0.05)*p*≤). Sample 4 was introduced as the superior treatment due to its suitable physical characteristics and clearer color changes at different pH. Sample 4 was introduced as the superior treatment due to its suitable physical characteristics and clearer color changes at different pHs, and in general, it can be said that the films prepared due to their sensitivity to pH changes can be used in smart packaging. .

5- Resources

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لیلا علیزاده[۱]، نفیسه جهانبخشیان*[۲و۳]، محمدحسین عزیزی[۴]، رضوان موسیوی ندوشن[۵]، مرتضی احسانی[۶]

۱- دانشجوی دکتری، گروه علوم و صنایع غذایی، دانشگاه آزاد اسلامی واحد تهران شمال، تهران، ایران.

۲-استادیار، گروه علوم و صنایع غذایی، دانشگاه آزاد اسلامی واحد تهران شمال، تهران، ایران.

۳- استادیار، گروه علوم و صنایع غذایی، دانشگاه آزاد اسلامی، واحد شهرکرد، شهرکرد، ایران.

۴- استاد تمام، گروه علوم و صنایع غذایی، دانشگاه تربیت مدرس، تهران، ایران.

۵- دانشیار،گروه علوم و صنایع غذایی،دانشگاه آزاد اسلامی واحد تهران شمال، تهران، ایران.

۶- استاد، پژوهشگاه پلیمر و پتروشیمی ایران، تهران، ایران.

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* مسئول مکاتبات:

jahanbakhshian.n@gmail.com

چکیده

در تحقیق حاضر ویژگی‌هایفیلم نشانگر پلی لاکتیک اسید و آنتوسیانین‌های استخراج شده از کلم قرمز و چغندر لبویی مورد بررسی قرار گرفت به طوری که نمونه‌های فیلم ۱ (پلی لاکتیک اسید فاقد آنتوسیانین)، ۲ (پلی لاکتیک اسید حاوی آنتوسیانین کلم قرمز)، ۳ (پلی لاکتیک اسید حاوی آنتوسیانین چغندر لبویی)و ۴ (پلی لاکتیک اسید حاوی آنتوسیانین چغندر لبویی و کلم قرمز) تهیه شدند و آزمون هایضخامت، حلالیت، خواص مکانیکی (میزان کشش پذیری، مقاومت به کشش، مدول الاستیک) و تغییرات مولفه های رنگیبر روی آنها صورت پذیرفت. نتایج نشان داد که اختلاف آماری معنی داری در ضخامت نمونه‌های فیلم ملاحظه نشد(p>۰/۰۵). بالاترین حلالیت متعلق به نمونه شاهد (فیلم ۱) و پائین ترین میزان حلالیت متلعق به نمونه ۴ بود.نتایج آزمون های مکانیکی نشان داد که بالاترین افزایش طول در شکست متعلق به نمونه های ۳ و۴ و پائین ترین افزایش طول در شکست متعلق به نمونه ۱ بود (p≤ ۰/۰۵). همچنین پائین ترین مقاومت به کشش و مدول یانگ متعلق به نمونه ۱ بود (p≤ ۰/۰۵). در تمامی نمونه‌های فیلم به جز نمونه ۱، با افزایش pH (تاpH=14)،مولفه رنگی*Lنمونه‌ها به طور معنی داری افزایش یافت (p≤ ۰/۰۵). همچنین در تمامی pHهای مورد بررسی، نمونه ۱ دارای بالاترین مولفه رنگی *Lو نمونه ۳ دارای پائین ترین مولفه رنگی *Lبود (p≤ ۰/۰۵).مولفه رنگی *a در نمونه ۳، در pH=1-6، روند افزایشی و سپس تا pH=14 روند کاهشی داشت(p≤ ۰/۰۵). در نمونه‌های ۲ و۴ در pH=1-13، روند کاهشی و سپس تا pH=14 روند افزایشی ملاحظه شد(p≤۰/۰۵). در نمونه ۳، تا pH=6، روند افزایشی و پس از آن تا pH=14 روند کاهشی ملاحظه شد(p≤۰/۰۵). در مولفه رنگی *b نمونه ۲، ابتدا در pH=1-12، روند کاهشی و سپس تا pH=14 روند افزایشی ملاحظه شد (p≤ ۰/۰۵). مولفه رنگی *b نمونه ۳ در pH=1-11، و در نمونه ۴ در pH=1-12، روند کاهشی و مجدد برای هر دو نمونه تا pH=14 روند افزایشی نشان داد(p≤ ۰/۰۵). نمونه ۴ به دلیل ویژگی های فیزیکی مناسب و تغییرات رنگی واضح تر در pH های مختلف به عنوان تیمار برتر معرفی شد.