



## Scientific Research

### Fabrication of smart biosensor based on starch nanocrystal and Dutch rose To detect chicken spoilage

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## ABSTRACT

## ARTICLE INFO

In this study, an optimal pH-sensitive intelligent film based on starch at the level of 1% w/v containing 0.1% potato starch nanocrystals and anthocyanin extracted from the Dutch rose plant (*Rosa hybrida*) at the level of 0.5% w/v was designed and used to determine the spoilage of chicken fillet at refrigerator temperature during 12 days of storage. The index of volatile nitrogen bases <sup>1</sup> and thiobarbituric acid <sup>2</sup> of chicken samples increased significantly during 12 days. The results showed that the inoculation of the extract in the biofilm changed the total population of bacteria and psychrophilic bacteria in chicken fillets to below detectable levels within 8 and 12 days, respectively. The present study showed that the color change of the smart film at the end of the storage day corresponds to the microbial growth pattern and also the increase in TBA, TVB-N, and pH, which is caused by the production of nitrogenous substances and alkaline compounds by mesophilic and psychrophilic bacteria. Therefore, the film of potato starch nanocrystals/potato starch containing Dutch rose extract can be used as an indicator of the freshness of the meat fillet.

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## 1- Introduction

Intelligent packaging can be considered advanced active packaging that, while controlling the packaging conditions, provides information about product quality during storage and distribution and is capable of performing intelligent functions such as detection, sensor capability, recording, tracking, communication. and applying scientific logic to increase safety and provide information and warnings about possible problems. The unique feature of this packaging is its ability to be related to food.]1[.

Indicators may be defined as a substance that indicates the absence or presence of another substance or the degree of reaction between two or more substances by means of characteristic changes, especially in color. Indicator color changespH It can be suggested to check volatile acidic or basic compounds and create an irreversible color change in appearance. Markers are the most widely used tools due to their non-destructive method, simplicity, low cost and easy detection with the naked eye. Chemical and biological changes in food products are one of the natural events. Contamination by microorganisms is also the main cause of food spoilage. Microbes can produce various chemical metabolites such asCO<sub>2</sub>, organic acids, alcohol (ethanol), hydrogen sulfide (H<sub>2</sub>S) and produce nitrogen-containing molecules that are proposed as target molecules suitable for indicators.2]

Anthocyanins are very unstable compounds vspH, light, oxygen, storage temperature, the presence of enzymes, sulfur dioxide, metal ions, co-pigments, the structure and concentration of anthocyanins and the presence of other compounds such as other flavonoids and minerals. Anthocyanins are

responsible for different colors of flowers and fruits. Phenolic compounds are plant-derived elements with several biological activities, especially antioxidant and antimicrobial abilities. Most importantly, phenolic compounds extracted from plants are safe. Therefore, it is possible to produce packaging films with the combination of anthocyanins to monitor food quality. In addition, anthocyanins as an important and sensitive indicatorpH They are used to evaluate food spoilage]3 [Among anthocyanin pigments, cyanidin-3- Glucoside is the main anthocyanin found in most plants. Green leafy vegetables, grains, berries, grapes and some tropical fruits have high anthocyanin content.. In addition, carrots, red cabbage and potatoes are rich in anthocyanin, which are used to prevent diseases. In acidic conditions, anthocyanins appear red and have a purple colorpH are neutral but with an increase pH They turn blue.4] Anthocyanin extracted from ripe blackberries has been used to develop a quality index to monitor the quality of fresh chicken meat during storage.]5[. Rose contains anthocyanin pigments, multivalent cyanidin and malvidin-glycoside. Rose contains sugar components linked by aglycones through glycosidic bonds]6[.

Starch is one of the most abundant and cheapest polymers in nature. The ability to form a starch film is attributed to the amylose content in it. The presence of side chains prevents intermolecular communication and weakens the film-making properties of polysaccharide, so branched amylopectin-based films are weak and fragile, while linear amylose molecules form strong and flexible films. ]7[. The use of essential oils, extracts and nano particles improves the mechanical properties, permeability and hydrophilicity of

starch films, and on the other hand, the use of these additives and sensitivity to factors such as pH. It causes the desired film to be considered as a smart packaging. Based on this, in the present research, Dutch rose extract was used as a rich source of anthocyanin to create a sensor sensitive to pH. Potato starch based film was used to monitor the freshness of chicken fillet.

## 2- materials and methods

### 2-1- Materials

Roses were purchased from the local market in Tehran, then dried and stored in a dark and dry place until use. Potato starch was obtained from Nano Alborz Pharmaceutical Company (Karaj, Iran). Other reagents used were of laboratory grade. All chemicals were purchased from Sigma-Aldrich (St. Louis, USA).

### 2-2- Extraction of rose plant extract

Aqueous extraction method was used to extract bioactive compounds [8]. For this purpose, the ethanol solvent is proportional to water 80:20 to volume 500 ml and with 50 g warm rose powder mixed and for effective extraction, from the ultrasonic bath at temperature 30 degrees Celsius for the duration 20 minutes were used. After filtering the extract with filter paper (Whatman, No 4 (using a freeze dryer) Armfield FT-33, Ltd., Ringwood, England) in temperature 55-degree Celsius for duration 24 hours under pressure 22-40 Pascal; The dry extract was stored in a dark and dry place.

### 2-3- Preparation of potato starch nanocrystals

Potato starch nanocrystals based on the method proposed by Zhou et al. (2016) were prepared [9]. 25 grams of potato starch, 250 milliliters of molar sulfuric acid 3/16 Molar mixture and suspension for the duration 7 days on the shaker at speed 120 rpm at temperature 1±40 degrees Celsius was placed. Then mix in 1000 rpm for duration 10 minutes centrifuge for a minute and washed with distilled water until pH equal to 7 reach

### 2-4- Preparation of potato starch film and sensitive to pH

To prepare the initial film formation solution, potato starch on the surface 1% (weight/volume), potato starch nanocrystals in 0.1 weight/volume percentage and glycerol in 0.5 % by weight/volume. At 100 ml of distilled water with stirring 700 rpm for duration 30 minutes is resolved. solutions by Ultra-Turra Digital in 10000 rpm for duration 2 minutes homogenized and in a refrigerated centrifuge (Sigma 8K, Germany) with rotation 3000 rpm for duration 5 minutes were placed for a minute to remove air bubbles from the solution [10]. Potato starch films with addition 30 milliliters of each film-forming solution in a plastic Petri dish diameter 80 mm and placing them in the oven with temperature 2±42 degrees Celsius for the duration 24 hours were made. The suitable film was chosen according to its appearance, transparency and flexibility [11].

The sensor films were prepared by using starch film forming solution and adding bioactive extract powder of rose petals. 0.5 Gram extract powder to 100 milliliters of film-forming solution and after complete mixing, mix with Ultra-Turrax Digital in the round 2000 for the duration 5 minutes. The mixture was homogenized. The resulting mixture is transferred to the petri dish and for a long time 24 hours in temperature 40 degrees Celsius to dry.

### 2-5- Oxygen permeability of the sensor film

To determine the oxygen permeability (OP) of the sensor film, a GDP gas permeability tester (CD-80335, Munich) was used.

became. Briefly, film on a stainless steel mask with an open test surface 0/0143 square meter was exposed to oxygen flow at temperature 24 degrees Celsius and 50% RH was placed]12[.

Four measurements were taken to determine the permeability and the average data was reported as OP rate (cm<sup>3</sup> μm/m<sup>2</sup> d kPa).

### 2-6- Mechanical properties of sensor film and potato starch

A Brookfield tissue analyzer (CT3, USA) was used to determine the mechanical properties of the sensor film, and mechanical analysis was performed.13[. The investigated mechanical properties included elongation at break (EB) and tensile strength (TS).

### 2-7- Preparation of chicken samples

Freshly purchased chicken samples from the Tehran market were placed on ice after preparation and transferred to the laboratory, and their microbial and chemical characteristics were determined within days.0, 4, 8 And 12 was determined

### 2-8- measurement pH Chicken samples:

To determine the pH of chicken samples from a laboratory pH meter (Jenway, 3510 England) Use became.10 Sample gram in 100 ml of distilled water was homogenized and the electrode of the device was placed in the mixture.

### 2-9- Measurement of thiobarbituric acid index (TBA) and volatile nitrogenous bases(TVB-N)

Thiobarbituric index was determined according to the method described by Jouki et al. (2014).]14[. It was determined by calculating the amount of malondialdehyde (mg/kg chicken sample). To determine the content TVB-N from The method described by Malle et al. (1987) was used] 15[ and the average measurement was reported. TVB-N as mg of nitrogen (N)/100 Gram of the sample was expressed.

### 2-10- Microbial analysis

25 Gram of the sample was prepared aseptically and to 225 ml of sterile peptone

water solution (0/1 percent) was transferred. Then mix for a while 2 Minutes were homogenized using a sterile blender. For microbial enumeration, samples 0/1 milliliters of serial dilutions (10:1, diluent) sample homogenates were spread in Kant agar plate culture medium. Total number of viable microorganisms (TVC) and cryogenic count (PTC) on standard Kant agar plate medium (PCA, Merck) at temperature 7 degrees Celsius for the duration 12 day were examined]12[.

### 2-11- Determine the color:

Color changes of the sensor film were measured using a Minolta colorimeter (CR-400, Japan). After measuring the brightness (L), redness (a) and yellowness (b) parameters, the total color difference (ΔE) of the film placed on the samples was calculated and the difference was recorded (equation 1). Also, the color changes of the coated film on the samples were visually checked.

(1)

$$\Delta E = \sqrt{(L - L_f)^2 + (a - a_f)^2 + (b - b_f)^2}$$

And  $L_f$ ,  $a_f$ , and  $b_f$  The values of the color parameters L, a and b are for the fresh sample]1 [.

### 2-12- statistic analysis

Statistical analysis of data using software 17/SPSS (version:21.0) and Excel is done, then homogeneity of data variance is done. In order to statistically compare the features of the film, first from analysis of variance (ANOVA), then to compare the averages from Duncan's multiple comparison test ( $p < 0/05$ ) was used and the results were presented as mean  $\pm$  standard deviation. Graphpad software was used to analyze the correlation evaluation results.

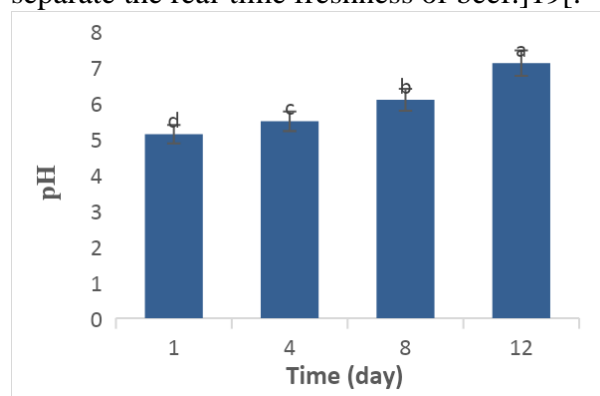
## 3- Results and discussion

### 3-1- pH Chicken:

The pH of the samples at the beginning of the storage period in the chicken sample is equal to 5/13 was that by increasing the holding time up to 12 Day It has 7/11 increased (Fig1).

Based on the obtained results, with increasing storage time, the pH of the chicken sample increased significantly ( $p < 0/05$ ). which is in agreement with previous studies]17[. The increase in pH in treatments can be due to the activity of microorganisms and the increase of volatile compounds such as: ammonia, trimethylamine and dimethylamine.]18[.

The result of this study is that there is a correlation between the change in the color of the packaging and the pH changes in accordance with the results expressed by Yan et al. (2019) who used litmus paper reagent to separate the real-time freshness of beef.]19[.



**Fig 1)** pH of chicken samples packaged with potato starch/ potato starch nanocrystals/Dutch rose extraction for 12 days. Different letters show significant difference at  $p < 0.05$

### 3-2- Index of nitrogen volatile bases( TVB-N and thiobarbituric acid(TBA)

#### Chicken sample

Nitrogen volatile bases (TVB-N) is a parameter that determines the compounds consisting of ammonia and primary, secondary and tertiary amines, and its increase is related to the activity of spoilage bacteria and related endogenous enzymes.]20[. The increase in TVB-N content is related to bacterial spoilage and endogenous activities]21[. In this way, volatile bases are produced by separating amines from amino acids by microbial enzymes ]22[. According to the instructions of the public health supervision office of the

country's veterinary organization, if the amount of tvb-n in chicken meat is more than 27 milligrams per 100g. If the meat is warm, the meat will be inedible. This amount if maximum 20- 21-24 And 25-27 milligrams per 100g. If it is hot, the consumption of meat will be optimal, edible and fast.]23[

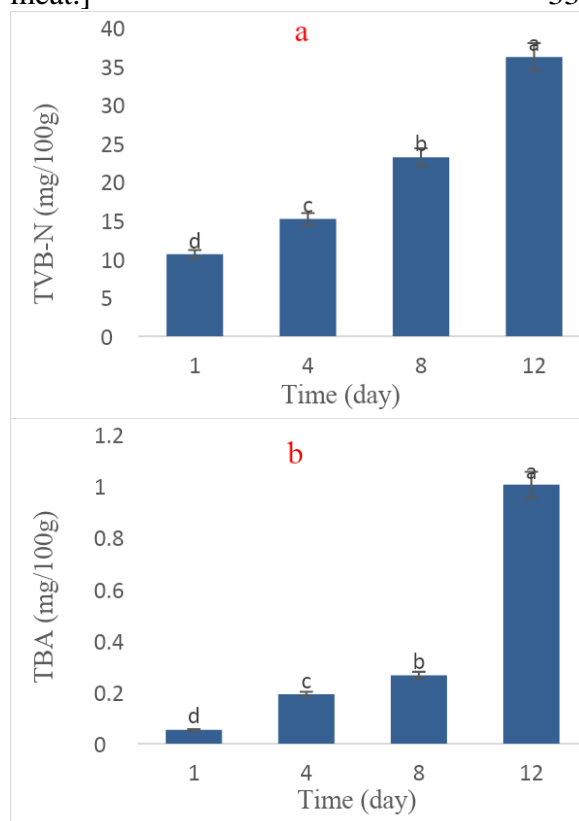
Amount of TVB-N in mg/100g Sample gram in chicken meat in fig2 it has been shown. Based on the obtained results, the amount of TVB-N increases significantly with the increase of storage time ( $p < 0/05$ ). This trend is in agreement with previous related research on other poultry species]24[. The minimum and maximum amount of TVB-N in chicken meat, respectively 10/64 mg in 100 gram sample on the first day and 36/19 mg in 100 sample grams per day]12 is. The increase in TVB-N during the storage period can be related to the activity of spoilage bacteria, the high activity of bacteria breaks down compounds such as trimethylamine oxide, peptides, and amino acids into volatile bases.]25[Ali Sahari in (2013) reported that the level of TVB-N in poultry meat if the amount is equal to mgN/100g 6/19 It is a sign of the freshness of the chicken]26[.

Ojagh et al (2010)]27[ reported that TVB-N in rainbow trout fillet coated with compd

Chitosan and cinnamon essential oil, chitosan-coated fillets and control fillets significantly increased during the storage period, however, until the end of the storage time (day16) the increase in the fillets coated with the combination of chitosan and cinnamon essence was lower compared to the fillets coated with chitosan and finally the control fillets. Due to the higher growth of bacteria in the control sample and the amount of volatile nitrogen bases is also higher, chitosan covered with anthocyanin had less volatile nitrogen bases, which can be related to the lack of growth of bacteria in this type of coating. Therefore, it can be said that coating preserves the quality of the samples during the entire storage period, and edible coatings act as antimicrobial substances and affect the amount of volatile bases. It can be concluded that coating is effective in reducing the volatile bases of chicken fillet samples during storage. Thiobarbituric acid is usually used to measure the oxidation state of meat foods]28[. Based on the obtained results, with increasing storage time, the amount of thiobarbituric acid increases significantly ( $p<0/05$ ). Minimum and maximum amount of thiobarbituric acid in chicken fillet, respectively 0/054 On the first day and 1/006 On the day 12 At 100 Gram is an example. (Shape 2). The presence of TBA in the process of spontaneous fat oxidation, during which peroxides are converted to aldehydes and ketones, depends on the content of secondary oxidation compounds.]29[. But the amount of TBA may not show the real level of lipid oxidation, because malondialdehydes can react with other components of chicken fillet. Among these compounds, amines, nucleotides and nucleic acids, proteins, amino acids and phospholipids can be mentioned. Such reactions depend to a large extent on the type of bird species]30[Buyn and colleagues in 2003, Mizan 2 Milligrams of malondialdehyde per kilogram of meat

have indicated the beginning of fat oxidation and the beginning of changes in the taste of chicken meat, while Teets and colleagues in 2008, the amount 3 have reported milligrams of malondialdehyde per kilogram along with oxidative spoilage in meat.32 ,31 .[

In the study of Yingyuad et al. (2006), they showed that the use of chitosan coating in pork meat stored at refrigerated temperature significantly reduces fat oxidation and increases the quality and shelf life of the meat.] 33[.



**Fig 2) (a) TVB-N, (b) TBA** of chicken samples packaged with potato starch/potato starch nanocrystals/Dutch rose extraction for 12 days. Different letters show significant difference at  $p<0.05$

### 3-3- Investigation of the total count of bacteria and psychrophilic bacteria

Based on the obtained results, with the increase of storage time, the logarithm of

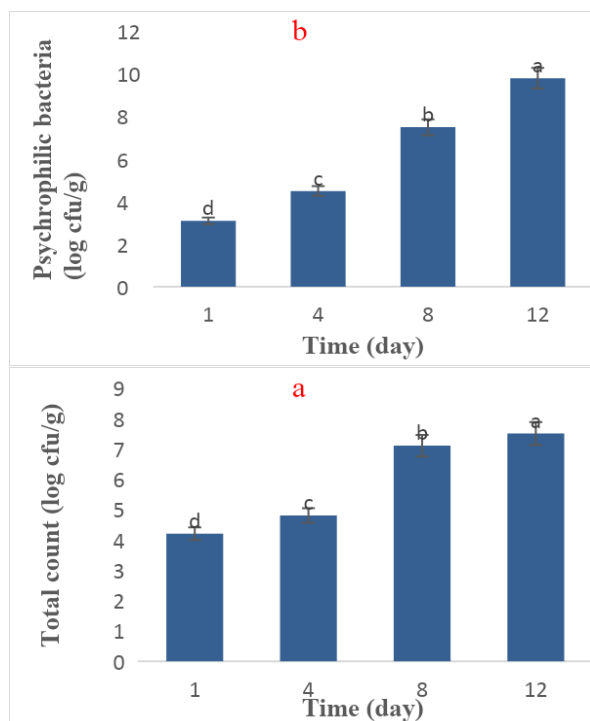
total bacterial count increases significantly ( $p < 0/05$ ). The minimum and maximum logarithm of total bacteria count in chicken fillet, respectively  $4/2$  on the first day and  $\log$  CFU//g)  $7/5$  On the day  $12$  is (Figure a3). In the current research, the amount of total bacteria on the eighth day of the storage period is  $\log$  CFU/g  $7/1$  It was found that the recommended limit was exceeded, so chicken fillet samples were used as spoiled samples on this day to investigate the color changes of the sensor film.

Freshly slaughtered carcass surfaces usually contain  $10^3$  to  $10^5$  There are bacteria in every square centimeter of carcass or one gram of meat, which due to poor storage conditions and favorable conditions for the growth of microorganisms, their number has increased rapidly and when their number reaches more than  $10^7$  In every square centimeter of the meat, spoilage will begin, in which case the surface of the meat will become cloudy and slimy and give off an unpleasant smell.<sup>34</sup> In the study of Yao et al. (2013), the antibacterial effect of biodegradable coatings on bacteria was confirmed<sup>35</sup>. A significant decrease in the total number of bacteria in the coated samples is due to the application of the coating, which acts as a barrier against the transfer of oxygen and leads to the inhibition of the growth of aerobic bacteria.<sup>36</sup>

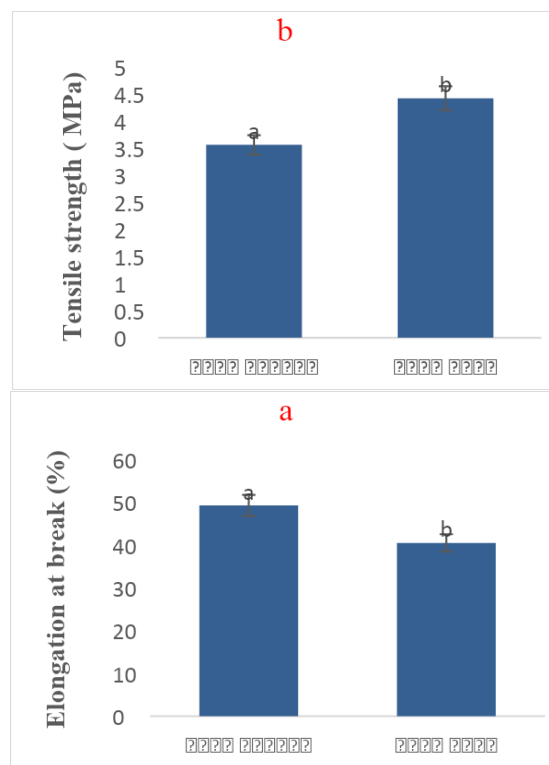
A high number of microbial load can be found in the raw product, which depends on the storage conditions and secondary contamination. *Aeromonas*, *Moraxella* and *Pseudomonas* are among the most important bacterial groups that were isolated from poultry meat<sup>37</sup>. The increase of the total microbial load for each treatment during the storage period depends on the level of compliance with the hygiene principles in the processing methods and the initial amount of bacteria. Increasing the temperature and increasing the duration of the storage period

causes the growth and proliferation of bacteria<sup>38</sup>.

Based on the obtained results, with the increase in storage time, the logarithm of the total count of psychrophilic bacteria in the chicken sample increases significantly ( $p < 0/05$ ). The minimum and maximum logarithm of the total count of psychrophilic bacteria in chicken fillet, respectively  $3/1$  on the first day and  $\log$  CFU//g)  $9/8$  On the day  $12$  is (Figure b3). The reason for the increase in microbial load at low temperatures can be due to the growth of cold-oriented bacteria. In the sample containing anthocyanins, due to the presence of anthocyanins as a preservative and also their antioxidant properties, they grew less than other treatments. The results obtained from the microbial counting experiments of cold-oriented bacteria were consistent with other results<sup>40</sup> ‘<sup>39</sup>. Cold-oriented aerobic bacteria such as *Pseudomonas* species are among the dominant bacterial groups in poultry and fish meat, which widely contribute to the spoilage of meat stored in aerobic conditions.<sup>41</sup> Allowable bacterial load for aerobic cold-loving bacteria ( $\log$  CFU//g)  $7$  It has been reported<sup>42</sup>. For example, chicken per day  $8$  The limit was exceeded. Gram-negative cold-loving bacteria are the main group of spoilage-producing microorganisms in aerobic conditions and at cold temperatures<sup>43</sup> ‘<sup>27</sup>. In this study, cold-oriented flora bacteria prevailed, and the main role of these bacteria in spoilage of samples is deamination of free amino acids and production of volatile nitrogenous compounds, which, in addition to reducing nutritional value, cause unpleasant odor and taste in them. These results were consistent with the results of other studies on the burden of cryogenic bacteria in other species during cold storage<sup>45</sup> ‘<sup>44</sup> [.



**Fig 3** (a) Total count, (b) Psychrophilic bacteria of chicken samples packaged with potato starch/potato starch nanocrystals/Dutch rose extraction for 12 days. Different letters show significant difference at  $p < 0.05$



**Fig 4** (a) Elongation at break (b) Tensile strength in potato starch film and pH-sensitive intelligent film. Different letters show significant difference at  $p < 0.05$

### 3-4- Investigating the mechanical properties of sensor film and potato starch film

The comparison results of the average elongation percentage when breaking the film containing potato starch and the film containing rose extract are shown in the graph below. Based on the obtained results, the percentage of elongation at break in the film containing rose extract is lower than the film containing potato starch, which statistically had a significant difference between the samples ( $p < 0.05$ ).

Also, the comparison results of the average tensile strength of the film containing potato starch and the film containing rose extract are shown in the graph below. Based on the obtained results, the amount of tensile strength in the film containing rose extract is higher than the film containing potato starch, which statistically had a significant difference between the samples ( $p < 0.05$ ).

The mechanical properties of composite polymers are among the properties that depend on the level of interactions at the interface of the compounds. In general, the establishment of appropriate interactions between compounds causes a significant improvement in the mechanical properties of films.<sup>46]</sup> The mechanical properties of films depend on the intermolecular forces of their constituent polymer chains, the ratio of constituent compounds, added additives and environmental conditions. Tensile strength is the maximum stress required to tear the film during the tensile test. Tensile strength is measured with a texture analysis device, which is based on the longitudinal stretch of the film up to the point of tearing. The term elongation at break is used if the ratio of the stretched length of the film to its original length before the point of tearing is It is



reported as a percentage, the term elongation percentage is also called tear resistance index.47] tensile strength and elongation at break for the produced film are respectively equal to  $0.37 \pm 3/56$  megapascal and  $2.02 \pm 49/33$  percentage and by adding rose extract to the film structure, film strength and elasticity, respectively  $0.31 \pm 4/42$  megapascal and  $3.89 \pm 40/56$  percentage was obtained, which was due to the change of the mechanical structure and the flexibility of the film structure with the increase of dry matter.

### 3-5- Examination of permeability to oxygen

Preventing the penetration of oxygen is also very important because it participates in chemical and microbial reactions that lead to spoilage of meat products in food packaging. The low oxygen permeability (OP) of packaging films plays an important role in preserving these products. As proven in previous research, due to the hydrophilic structure of saccharide-biopolymers and high hydrogen bonds and gel network, polysaccharide layers are usually good barriers against oxygen and non-polar volatile substances.48] In this research, the sensor film has an OP equal to  $1/19 \pm 14/30$  cm<sup>3</sup>/m<sup>2</sup> was d kPa, which indicates the suitable gas barrier of this film. The results obtained from the mechanical and oxygen permeability test showed that this film as a pH-sensitive sensor is able to show a suitable and flexible structure with a suitable gas barrier.

### 3-6- Investigating the color characteristics of the biosensor on the chicken sample

Another evaluated index was colorimetry, which was recorded using a Minolta colorimeter (CR-400, Japan) and the change in a specific color spectrum indicated the contamination of the sample. The numbers obtained from the colorimeter in the healthy sample correspond to the first day of storage and the numbers obtained in the spoiled

sample correspond to the eighth day of storage.



**Fig 5)** Color changes in the intelligent pH indicator PS-films on the chicken fillets after 0 and 8 days of storage at 4 °C for fresh and spoiled chicken, respectively.

The amount of L\* index, which indicates the brightness of the sample, was lower in the healthy chicken meat sample than in the rotten sample. The value of the a\* index, which indicates the redness of the sample, in healthy chicken meat shows a higher numerical value compared to rotten chicken meat, so as a result, it can be said that the sample is red in healthy chicken meat according to the numbers obtained from the colorimeter. is more and the higher values of this index can be attributed to the freshness of the chicken meat sample. The amount of b\* index indicates the yellowness and blueness of the sample. This index showed a lower number in healthy chicken meat than in rotten chicken meat. Chicken meat is considered a food product rich in protein and the growth of microorganisms is one of the most important reasons for its spoilage during storage. As a result of the growth of microorganisms, The proteins in the meat are converted into biogenic amines, which increase pH Meat and quantity TVB-N can be

]49]. Based on the obtained results, it can be concluded that the spoilage of the chicken sample causes the intended packaging to decrease significantly due to the spoilage in the chicken sample, indicating a decrease in the amount of anthocyanin, and the results of TVB-N have been reported. In the previous sections, it was shown that this index increases by taking into account a measure of spoilage of chicken samples, and as a result, there is a negative correlation between the  $a^*$  index and TVB-N. On the other hand, this result was associated with the breakdown of the product by the enzymes secreted by microorganisms, the breakdown of proteins and the production of biogenic amines.

TVB- N, which is the result of the growth of special spoilage microorganisms, its amount increases and its concentration increases in the empty space of the packaging, which results in an increase in the pH of the packaging atmosphere. with increasing TVB-N and as a result the pH increases, the color of the indicator inside the package changes and conveys information from inside the package]50[. In another study in which basil leaf extract was added to the gelatin film, this colorimetric index decreased significantly with the passage of time, and the comparison of this index with TVB-N showed that there is a negative correlation between these two parameters.]51[. Also, the addition of Hasan Yusuf extract to the gelatin film and the investigation of salmon spoilage showed that the TVB-N index of 9/3 To 55/4 mg in 100 Gram sample during 16 The storage hours at room temperature increased while the  $a^*$  index decreased during this period of time]50[. Changes in the  $\Delta E$  index compared to the TVB-N index can be a suitable factor for evaluating the spoilage of chicken samples. In this research, it was determined that by changing this index in chicken 10/64

mg on the first day and 36/19 milligrams per day 12 At 100 g and change index  $\Delta E$  of 0 To 2/67 On the eighth day, it has a direct relationship with the color index  $\Delta E$ . Accordingly, in another study, the addition of sour tea petal extract to starch/polyvinyl alcohol film and pork packaging showed that the color index ( $\Delta E$ ) increased along with the TVB-N index. so that during 24 The first hour of this index 7/25 To 11/28 mg in 100 Warm and after 36 clock to 15/69 mg in 100 Gram also increased the color index  $\Delta E$  of 0 To 12 and after 36 clock to 15 increased] 50[.

Kraig et al. (2013) used a polyaniline (PANI)-based indicator film produced using a polystyrene substrate to detect fish spoilage, which was studied through visible color changes with changes in TVB-N levels during fish spoilage. given. The results showed that the color changes, in terms of total PANI color difference, have a good relationship with the TVB-N level of the fish. Apart from TVB-N, tests on fish milk samples showed that there is a good correlation between the PANI film response and the microbial growth patterns in the fish samples, especially the change of the microbial population (acceptable total number of TVC) and *Pseudomonas*) and with increasing population. microbial, the color of the film gradually changed from green to blue. These responses provide the possibility of rapid control of fish spoilage at different times with constant temperature or with temperature fluctuations] 51[.

**Table 3-1:** Color change of intelligent film during storage period of chicken fillets samples

Samples	L	a	b	ΔE
Fresh chicken	17.32±0.32 <sup>a</sup>	10.95±0.16 <sup>a</sup>	15.97±0.28 <sup>a</sup>	30.87±0.31 <sup>a</sup>
Spoiled chicken	19.11±0.58 <sup>b</sup>	8.91±0.16 <sup>b</sup>	16.26±0.84 <sup>b</sup>	28.2±0.26 <sup>b</sup>

Data represent mean ± standard deviation. The difference in superscript letters in each column indicates a significant difference at a probability level of 5%.

### 3-7- Assessment Correlation between pH, the degree of microbial, chemical and color parameters of the sensor

To investigate the relationship between the sensor's color parameters and chicken meat spoilage values pH, TVB-N And the total bacteria and the amount of cold-oriented bacteria were measured. The results of this survey are given in the table below.

By drawing correlation graphs, a significant relationship was observed between the color parameters of the indicator and the spoilage characteristics of the chicken meat sample.

**Table 3-2:** The results of the relationship between the color parameters of the sensor and the spoilage parameters of chicken meat

Color parameters			Corruption parameters
b*	a*	L*	
r <sup>2</sup> =0.9568	r <sup>2</sup> =0.9509	r <sup>2</sup> =0.9519	pH
r <sup>2</sup> =0.9585	r <sup>2</sup> =0.9526	r <sup>2</sup> =0.9536	TVB-N
r <sup>2</sup> =0.9212	r <sup>2</sup> =0.9188	r <sup>2</sup> =0.9192	TVC
r <sup>2</sup> =0.9865	r <sup>2</sup> =0.9828	r <sup>2</sup> =0.9834	PTC

### 4-total resulting

In this research, a smart film based on starch and potato nanocrystals containing rose bioactive compounds was prepared and examined on a chicken fillet sample. Sensor color changes due to pH changes caused by microbial and chemical spoilage of chicken fillet were clearly identifiable, so that a significant difference in sensor color was

Considering the positive numbers, it can be said that there is a direct relationship between color parameters and corruption factors. Also, considering that the values obtained in numbers 1 are close as a result of communication There is a high linearity between the color parameters of the sensor and spoilage factors, as well as the spoilage parameters of chicken samples with increasing storage time at temperature 4 degrees Celsius increased.

created both visually and with a digital colorimeter. With the progress of spoilage of chicken fillet during storage, with increasing values of TVB-N, TBA and pH parameters, pH-sensitive smart films showed a distinct color change from light green to dark green. which promises the possibility of commercial use of this smart sensor as a pH-sensitive indicator in the detection of corruption of

protein samples. In summary, pH-sensitive smart films based on potato starch/Nanocrystals of potato starch and Dutch rose

extract can be used as an indicator of chicken freshness and shelf life improvement.

### 5- Resources

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## ساخت حسگر زیستی هوشمند بر پایه ی نانوکریستال نشاسته و رز هلندی جهت تشخیص فساد مرغ

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### چکیده

### اطلاعات مقاله

در این مطالعه، یک فیلم هوشمند بهینه حساس به pH بر پایه نشاسته در سطح ۱٪ وزنی/حجمی حاوی ۰/۱٪ نانوبلورنشاسته سیب زمینی و آنتوسیانین استخراج شده از گیاه رز هلندی (*Rosa hybrida*) در سطح ۰/۵٪ وزنی/حجمی طراحی و برای تعیین فساد فیله گوشت مرغ در دمای یخچال در طول ۱۲ روز نگهداری مورد استفاده قرار گرفت. اندیس بازهای فرار نیتروژنی<sup>۱</sup> و تیوباریتوریک اسید<sup>۲</sup> نمونه مرغ به طور معنی داری در طول ۱۲ روز افزایش پیدا کرد. نتایج نشان داد که تلقیح عصاره در بیوفیلم، جمعیت کل باکتری‌ها و باکتری‌های سایکروفیل را در فیله‌های مرغ به ترتیب طی ۸ و ۱۲ روز به زیر سطوح قابل تشخیص تغییر داد. مطالعه حاضر نشان داد که تغییر رنگ فیلم هوشمند در پایان روز ذخیره سازی با الگوی رشد میکروبی و همچنین افزایش TBA، TVB- N و pH مطابقت دارد که ناشی از تولید مواد نیتروژنی و ترکیبات قلیایی توسط باکتری های مزوفیل و سایکروفیل است. بنابراین، فیلم نانوبلورهای نشاسته سیب زمینی/ نشاسته سیب زمینی حاوی عصاره گل رز هلندی می‌تواند به عنوان شاخص تازگی فیله گوشت استفاده شود.

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