A Visual pH Indicator through Purple Cabbage Dye for Freshness Test of Venison

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Abstract: A visual pH indicator through purple cabbage dye is selected to test the freshness of venison. Chitosan and cassava starch of an equal weight were used to prepare the film-forming matrix of the indicator. The crystallization of natural purple cabbage dyes with a weight ratio of 5%, 10%, 20% and 40% were added to the matrix, respectively. The pH color test showed that the natural purple cabbage lyophilized powder with a weight ratio of 40% was the best for the pH indicator, which was used to test the freshness of venison stored at 4°C. The total bacterial count, volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) of venison were tested through an experiment. When stored at under 4°C, a significant color change of the indicator from pink to blue-green was shown when the total amount of volatile basic nitrogen (TVB-N) (22.78 mg/100 g) exceeded the critical value (20 mg/100 g). The test result showed that the indicator was very sensitive to pH value and it would help customers identify the freshness of venison.

Keywords: Visual pH indicator; purple cabbage dye; TVB-N; TBA; venison

1 Introduction

Food safety is continuously evolving in response to growing challenges in the modern society. Every year, a growing amount of edible food is lost within the entire food supply chain. Microbial growth in food products results in a reduction of food shelf-life and an increase in the risk of food-borne diseases [1]. This offers a unique opportunity to the packaging industry to provide innovative solutions to address the changing demands in the food industry. With a continuous development of materials science, computer technology, modern control technology, artificial intelligence and other related technologies, the traditional manufacturing industry is being constantly changed to intelligent manufacturing. In the future, with the development and an in-depth integration of new technologies such as printed electronics, RFID (radio frequency identification), flexible display and 5G communications, especially the rapid development and application of various sensing technologies such as electronic tags and QR codes, a brand-new transformation and upgrading will be brought to the traditional packaging industry, so as to realize the development of everything on the internet. Smart packaging is widely used in all fields and industries, such as food, beverages, electronic goods, medicine and daily necessities [2–10]. The packaging industry is constantly trying to enhance product safety by acquiring new technologies. Therefore, among the packaging industry, retailers, consumers and the food industry, there is a special interest in developing a device that is simple, low-cost, rapid, reliable, non-invasive and non-destructive to evaluate real-time freshness of food products [11–13]. An alternative concept to address this requirement is the development of smart packaging indicator labels to monitor freshness status of food products, which indicate freshness by detecting the amount of CO2 gas, volatile nitrogen compounds and H2S produced during meat spoilage and specific color changes caused by them. As an effective method, freshness indicators are widely used in food freshness...
Meat freshness indicators are generally divided into pH value, volatile nitrogen, microbial, hydrogen sulfide sensitive indicators and so on [14,15].

Food spoilage process occurs rapidly with the microbial growth and creation of amines. An increase in the concentration of TVB-N (total volatile basic nitrogen) in meat products caused by the microbial production of biogenic amines is known to indicate the spoilage and the creation of products potentially hazardous to health. Malondialdehyde (MDA), an oxidized product of unsaturated fatty acids in meat, is used by thiobarbituric acid (TBA) to produce colored compounds [16]. The absorbance of colored products at 523 nm and 600 nm can be measured to test the freshness of meat products. The higher the TBA value is, the higher the lipid peroxidation degree will be, and the wider the range of corruption will be. Here, we combined two methods, namely total volatile basic nitrogen (TVB-N) and thiobarbituric acid method (TBA) to the test of venison spoilage. Through a comparison of these two methods, we can judge the accuracy of the indicator. The test method is not only simple, convenient and rapid, but is also cost-effective [17].

At present, there are some sensors that can be used to detect food spoilage, some of which are technologically advanced but made of materials that are not safe for human health, so they need to be kept separate from food [18]. Many people detect meat spoilage through gas sensors. Despite that they are rapid, they are relatively more costly and difficult to be applied to meat package. To solve this problem, the indicator labels presented in this paper are made of fully edible components/materials. In this study, chitosan and cassava starch are used as film-forming substrates, both of which are non-toxic and harmless materials for food. Compared with other kinds of composite membranes, they can be more safely applied to the detection of venison freshness. Chitosan is of good film-forming properties, which is a very important raw material for preparing food-grade packaging materials [19]. Many studies have shown that chitosan is of excellent bacteriostasis and anticaner properties, which has been widely used in daily food chemical industry and medical care. Chitosan and starch are polymers that can be obtained from renewable sources, which are of good film-forming properties and many applications in food industry, such as active and smart-packaging, meanwhile they can also be used to monitor and inform consumers about food conditions in real-time [20,21]. However, with the excellent mechanical properties and compatibility of chitosan, the mechanical properties and stability of the film can be improved to a certain extent by mixing chitosan with cassava starch and preparing the mixed film by adding some plasticizers. For example, Mara et al. prepared a mixed membrane with chitosan and cassava starch as the film-forming matrix and potassium sorbate (KS) as the bacteriostatic agent for salmon preservation, between which a good compatibility is shown in the results [22]. The addition of chitosan reduces the permeability of water vapor and improves the solubility of starch film. At the same time, the mechanical properties of the fresh-keeping film are improved when the ratio of them is 1:1.

In this paper, a freshness indicator based on purple cabbage dye is proposed to detect the spoilage of venison. Compared with other freshness indicators, this indicator has advantages including a large production capacity, a low cost, convenient use and no additional technical support. When venison spoils, the color of the indicator label will change from pink to blue-green. The result showed that the food freshness indicator label prepared with 40% of natural purple cabbage dye as the pH indicator had advantages including sensitivity, accuracy, safety and reliability to the changes of venison freshness compared with other concentrations.

2 Materials and Methods
2.1 Materials
Anhydrous ethanol, hydrochloric acid, glacial acetic acid and chitosan with 90% deacetylation were obtained from Tianjin Yongda Chemical Reagent Co., Ltd., China. Glycerin, purple cabbage and cassava starch were obtained from Carrefour Supermarket.

2.2 Preparation of Freshness Indicator Label
Chitosan (2 g) was dissolved in 2% acetic acid (100 ml) solution, stirred continuously for 30 min in
an environment with a constant temperature (60°C) until it completely dissolved, so as to prepare 2% chitosan solution (100 ml). Cassava starch (2 g) was heated and stirred and gelatinized in distilled water (100 ml) to prepare 2% starch solution. The two components were mixed according to the volume ratio of 1:1, then a certain amount of glycerol was added and stirred in a magnetic stirrer for 15 min to prepare the film-forming solution. Purple cabbage lyophilized powder with a purity of 247.4 mg/L was prepared by removing acidified ethanol with extractant in a rotary evaporator and freeze-drying in vacuum. Chitosan/cassava starch solution containing purple cabbage dyes was prepared by adding 5%, 10%, 20% and 40% (W/W) freeze-dried purple cabbage powder into 50 ml of chitosan/starch film-forming solution, whose pH value was adjusted to 5. After 15 min of magnetic stirring, the bubbles on the solution were removed by ultrasound. Finally, 50 ml of solution was dried (24 h) in a culture dish with a diameter of 90 cm, and four groups of indicators with purple cabbage dyes of different concentrations were prepared. The indicators were cut into suitable sizes for subsequent tests.

The cutting tools and cutting board were wiped with absolute ethanol and irradiated continuously under an ultraviolet lamp for 30 min. The freshness indicators with purple cabbage of different concentrations were cut to 1 cm × 1.5 cm. The packaged samples were refrigerated at 4 ± 1°C to simulate the selling environment for preservation in supermarket. The quality of venison samples was recorded every two days, and each index was tested for three times.

2.3 Characterization

Because the substance is of absorption characteristics for infrared radiation with different wavelengths, the freshness indicators of purple cabbage dye with different concentrations were characterized based on infrared spectroscopy, and the molecular structure as well as chemical composition of those indicators were analyzed. The measurement range was 500–4000 cm⁻¹. Purple cabbage is rich in anthocyanins, whose structure at different pH values is shown in Fig. 1 [23]. Anthocyanins mainly exist in the form of cations lacking electrons, which rarely exist in a free form in natural world. Most of them combine with sugars and acidic substances to form glycosylation and acylation. When pH > 7.0, ionized quinone bases (A⁻ or A₂⁻) and chalcones are formed because of proton electron transfer reaction of anthocyanins.

![Figure 1: Scheme of the pH-dependent structural interconversion among dominant forms of anthocyanidins in aqueous solution](image)

2.4 Determination of pH Value and the Number of Colonies

Microbial contamination was a main cause of venison spoilage. According to Standard GB 4789.2–2016, the total number of colonies during venison storage was determined. Meanwhile, 10 g of venison
samples were mixed into minced meat and placed in a beaker, into which 100 ml of potassium chloride solution was injected. A calibrated pH meter was used to determine the pH value of samples. The testing process was carried out according to Standard GB 5009.237–2016. Each group of experiments were conducted for three times to get the mean value.

2.5 Determination of Total Volatile Base Nitrogen (TVB-N)

TVB-N in venison samples was determined through an automatic Kjeldahl apparatus. 30 ml of boric acid solution was injected into the apparatus as the receptor solution and the distillation time was set as 180 s. Firstly, the reagent blank was determined and the blank value was obtained. 10 g of the minced venison sample was put into a distilling tube and then mixed with 75 ml of distilled water. The sample was shaken for 30 min until it was evenly dispersed and then 1 g of magnesium oxide was added. The distilling tube was connected to a distiller immediately. A 250 ml conical bottle was used as the receiving cup in the waste liquid receiving tank. 10 drops of methyl red-bromocresol green mixed indicator was added and the automatic Kjeldahl apparatus was started. After operation, 0.1 mol/L standard hydrochloric acid was used to titrate the receptor solution in the receiving cup until the solution was blue-purple in color. Then the content of TVB-N in the sample was calculated through formula (1) [24].

\[
X = \frac{(V_1 - V_2) \times c \times 14}{m} \times 100
\]

where:
- \(c\): the concentration of hydrochloric acid solution, mol/L
- \(m\): the quantity of the sample, g
- \(V_1\): the volume of hydrochloric acid used for testing samples, mL
- \(V_2\): the volume of hydrochloric acid used for blank sample, mL
- \(X\): the content of TVB-N in the sample, mg/100 g.

2.6 Determination of Thiobarbituric Acid (TBA)

The value of thiobarbituric acid (TBA) is related to the degree of lipid oxidation. The venison (5 g) was grounded into minced meat and placed into a 100 ml conical bottle, into which 7.5% trichloroacetic acid (25 ml) was injected. Then the bottle was shaken to mix the sample uniformly and a piece of double-layer filter paper was used to filter the sample twice. The supernatant (5 ml) was extracted from the filtrate and injected into the same volume of 0.02 mol/L thiobarbituric acid (TBA) for a 40-min water bath at 100°C. Centrifugated at 1600 r/min for 5 min after the filtrate was cool. The absorbance value of supernatant at 532 and 600 nm were measured, and parallel experiments as well as blank experiments were completed. Then the content of TBA in the sample was calculated through formula (2).

\[
X = \frac{A_{532} - A_{600}}{155 \times 10} \times 72.6 \times 100
\]

where:
- \(X\): TBA value of the sample, mg/100 g
- \(A_{532}\): Absorption value at 532 nm
- \(A_{600}\): Absorption value at 600 nm.

2.7 Color Variations of Freshness Indicator during Spoilage of Venison

The freshness indicators of venison stored for different numbers of days were taken out and their color was recorded with a portable chromatic meter. The \(L^*, a^*, b^*\) of the indicators were obtained as the first color \((L_1^*, a_1^*, b_1^*)\). The chromatic aberration of each group was compared with that of D0, whose initial value was \(L_0^*, a_0^*, b_0^*\). The chromatic aberration of each group was compared with that of D0, whose initial value was \((79.40, 73.97, 65.45, 57.54), a_0^*(3.93, 16.04, 21.31, 23.37)\) and \(b_0^*(2.90, 4.22,\)
–6.23, –3.63). The value of \( \Delta E \) was calculated through formula (3).

\[
\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}
\]

where:

\( \Delta E \): The chromatic aberration between the two colors, nbs

\( L_1^* \): Lightness value of the first color

\( L_2^* \): Lightness value of the second color

\( a_1^* \): The red/green value of the first color

\( a_2^* \): The red/green value of the second color

\( b_1^* \): The yellow/blue value of the first color

\( b_2^* \): The yellow/blue value of the second color.

3 Results and Discussion

3.1 Fourier Transform Infrared Analysis

As is shown in Fig. 2, the curve was a chitosan/cassava starch blank group, and the curve form of b to e was an infrared spectrum of the indicators of chitosan/cassava starch with 5%, 10%, 20% and 40% of purple cabbage dye, respectively. Compared with the blank group, the N-H and O-H bond-stretching vibrations were found to be 3281 cm\(^{-1}\) in a group, which resulted in a wider overlapping and multiple absorption peaks. The absorption peak moved to 3288 cm\(^{-1}\) after purple cabbage dye was added, which was related to the formation of hydrogen bonds in phenolic hydroxyl groups between purple cabbage dye and hydrogen atoms in chitosan. The absorption peaks of CH\(_3\) and CH\(_2\) appeared at 292 cm\(^{-1}\), and those at 2851 cm\(^{-1}\) were caused by C-H stretching vibration. The two peaks moved to 2932 cm\(^{-1}\) at a high wave number, and those at 2987 cm\(^{-1}\) were caused by the stretching vibration of -CH\(_2\) and -CH bonds after purple cabbage dye was added. Peaks at 1646 cm\(^{-1}\) (C=O bond-stretching vibration) and 1556 cm\(^{-1}\) (N-H bond-stretching vibration) were the characteristic peaks of chitosan. The magnified Fig. 2 showed that 1152 cm\(^{-1}\) and 1077 cm\(^{-1}\) were caused by the C-O bond-stretching vibration of C-O-H and C-O-C groups in gelatinized starch, which were the characteristic peaks of starch. The addition of purple cabbage led to a new peak at 1103 cm\(^{-1}\), which was caused by the presence of anthocyanin [25]. The peak of the indicator at 1077 cm\(^{-1}\) might be weakened due to the decrease of C-O-C bond energy, which would lead to changes in the mechanical properties of chitosan/cassava starch indicator label [22]. 816 cm\(^{-1}\) was the characteristic absorption peak of C-O-C functional group on anthocyanin b ring. The absorption peak of chitosan/cassava starch indicator label with purple cabbage dyes between 810 cm\(^{-1}\) and 930 cm\(^{-1}\) increased obviously, and some bonds could be enhanced, which might be due to the adjacent substitution of aromatic rings [26].

![Figure 2: Fourier transform infrared (FTIR) of freshness indicator](image)

In conclusion, there was a very good compatibility between purple cabbage dye and chitosan/starch film-forming matrix. The mechanical properties of the indicator label were changed by intermolecular
forces, but its chemical composition was not affected.

### 3.2 Analysis of pH Value and the Number of Colonies

Acidified ethanol was used as an extractant to extract purple cabbage dye. Two groups of freshness indicators with different pH values were made, which was adjusted between 3 and 12 respectively in each group. The changes in indicator color of different purple cabbage dyes at different pH values were shown in Fig. 3. We could see that the freshness indicators changed from pink in acidic conditions to green in alkaline conditions.

![Figure 3: Color change of indicators in different pH conditions](image)

As is shown in Fig. 4, it could be seen that the pH value of venison samples decreased with the increase of storage time in the first 6 days and declined to the lowest value of 6.15 on the 6th day. The pH value began to rise again from the 6th to the 12th day. The reason for the pH that decreased first and then increased was that in the early storage period, the muscle glycogen underwent glycolysis and lactic acid was produced, whose production and accumulation made the pH value of venison decrease. In the later storage process, the life activity of some spoilage bacteria in venison increased, which made the protein of venison decompose into alkaline amines or ammonia, so that the pH value of venison rose obviously.

![Figure 4: Potential of hydrogen (PH) changes during venison spoilage](image)

Fig. 5 shows the changes in the total number of colonies in venison during spoilage, whose gradual rise was seen in this test with the increase of storage time. The initial total number of colonies was 3.25 lg CFU/g, which met the first freshness standard. On the 4th day, the total number of colonies was 4.34 lg CFU/g, and on the 8th day, it was 5.87 lg CFU/g, which was the upper limit of the second freshness standard. On the 10th day, the total number of colonies was 6.76 lg CFU/g, which exceeded 6.00 lg CFU/g. The venison sample had an obvious odor and became soft and sticky.
Figure 5: Changes in the number of colonies during venison spoilage

3.3 TVB-N Analysis

The variation of TVB-N value during venison spoilage is shown in Fig. 6. The TVB-N value was 5.32 mg/100 g at the beginning. After the 10<sup>th</sup> day, it reached 22.87 mg/100 g, which exceeded the upper limit of 20 mg/100 g for meat products [27]. Therefore, it could be concluded that the venison spoiled completely on the 10<sup>th</sup> day. The reason for the increase of TVB-N was that proteins reacted with microorganisms and enzymes to decompose into volatile-base nitrogen, which was also one of the most important reasons why TVB-N could be used to measure whether venison was fresh or not.

Figure 6: Correlation between TVB-N and stored time

3.4 TBA Analysis

The value of TBA in the process of venison spoilage increased with the storage time. The initial TBA value of venison was 0.27 mg/kg, whose increase was due to lipid oxidation caused by bacterial rancidity.

Figure 7: Correlation between the TBA concentration and stored time
The TBA value increased slowly in the first 8 days, and the indicator color gradually changed from pink to purple red. The venison completely spoiled when the TBA value was higher than 1.0 mg/kg. According to Fig. 7, the TBA value was 0.84 mg/kg on the 8th day and 1.31 mg/kg on the 10th day, which indicated that the venison spoiled. Corresponding to the TVB-N value in Fig. 6, it showed that after the 8th day, the venison had begun to decay and bacteria were seriously breeding.

3.5 Color Variations Analysis

Fig. 8 showed that there was little change in the color difference of the freshness indicators with 5% and 10% dye during the application process, and the color resolution of the indicator labels was not high. This might be due to the uneven flow of dyes or their low concentration during the preparation of the indicators, resulting in an indistinct effect in the remaining two groups of dyes. The value of freshness indicators with a concentration of 20% and 40% was significantly higher than that of D0 during refrigeration storage, and the color difference of the indicator labels increased significantly from the 8th day of storage, showing a significant upward trend in the later period, which coincided with the upper limit of the second freshness standard for the total number of bacteria on the 8th day. At this time, the freshness indicators with a concentration of 20% and 40% reached the upper limit of the second-grade freshness standard on the 8th day, whose color was blue-green. According to the TVB-N numerical analysis of venison during deterioration in Fig. 6, it could be concluded that the venison completely spoiled on the 10th day, whose TVB-N exceeded the upper acceptable limit of consumers. On the 10th day, the color difference of each group relative to D0 was 5.59, 13.47, 24.91 and 32.23, indicating that the label color was between blue-green and yellow-green.

Figure 8: Chart of color change in food freshness indicator label

Amino acids and other nutrients in the protein of venison will be decomposed into substances including ammonia and volatile alkali during spoilage, which will increase the pH value of the packaging environment and indicate the color change of the indicator. According to the research, when $\Delta E > 6NBS$, people could distinguish color clearly through vision, that is, different colors could be distinguished visually, and the probability of perception was 100%. Two groups of freshness indicators with 5% and 10% of dyes did not change significantly during the whole process of venison spoilage. Fig. 8 shows that the color difference increases with the increase of dye concentration. When the dye concentration was 40%, the color difference reached the maximum, which was 32.23. The overall lightness value of the indicator was higher with a dye concentration of 5% and 10%. It was not easy to compare and observe the color, which was inconvenient to recognize. The lightness value of the indicator with a high concentration of 20% and 40% was relatively low, and the color was deeper, which was beneficial to the indicator effect. Therefore, it was concluded that the freshness indicator with an addition of 40% dye solution had the best effect on the freshness index of venison spoilage process.

4 Conclusion

We investigated a natural plant dye, which was taken as a chromogenic agent, to make new indicators
for the testing of the freshness state of venison. Purple cabbage rich in anthocyanins was selected as the raw material for natural dye extraction. Chitosan/cassava starch was used as film-forming matrix to prepare intelligent indicators for venison freshness. Purple cabbage indicators with different concentrations were used to detect the freshness of venison at 4°C. The results showed that the measured value of various quality indicators exceeded the national standards on the 10th day, and the venison spoiled. At this time, the freshness indicator labels with a concentration of 40% showed blue-green color. In this way, pH indicators of safe materials were innovative materials that could be used to provide a dynamic feedback to consumers about the quality of food products.

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