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Anthocyanin based sensory pad for determination of chicken meat spoilage

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ABSTRACT

Production and consumption of chicken meat has increased considerably with the increasing world population, and will continue to increase, due to its health benefits and its economic value. Chicken meat is a sensitive food due to its characteristics and microbiological load. Traceability is very important to increase food safety and biosecurity. Freshness indicators are often used to monitor the presence of metabolites produced by microorganisms, mostly functioning through the colour change of the indicator. A developed natural, edible, biodegradable, purple coloured pH sensitive sensory pad was put inside a chicken meat package to observe the chicken meat spoilage with colour change due to pH changes in the meat.

KEYWORDS

anthocyanin, intelligent packaging, microbiology, chicken meat, red cabbage

1. INTRODUCTION

The consumption of chicken meat and its products has increased due to their higher protein content, less fat, cholesterol, and excellent digestibility. Besides its high nutritional value, the

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water activity (0.98–0.99) and pH value of raw chicken meat are also suitable for the growth of microorganisms. The pH value of chicken breast and the pH value of chicken thigh are in the range of 5.7–5.9 and 6.4–6.7, respectively. Although skin plays a role as a physical barrier for microorganisms, it also contains many microorganisms. It was reported by Alvarez–Astorga et al. (2002) that hygiene practices, storage time, and temperature during processing affecting microorganism levels in chicken meat.

Some epidemiological studies indicate that chicken meats are one of the important causes of food infections and intoxications (Mulder, 1996). Thus, chicken meat must be packaged appropriately and delivered to consumers without any change in quality. Total aerobic mesophilic bacteria (TAMB), coliform bacteria, *E. coli*, *S. aureus*, psychrophil bacteria, and yeasts and moulds, which are included in the microbial flora of chicken meat, are considered as indicators and deteriorating microorganisms that determine hygienic quality (Blank and Powell, 1995). Freshness indicators for food products present information about their quality or freshness according to chemical changes or microbiological changes. Freshness indicators are often used to monitor the presence of metabolites produced by microorganisms. These metabolites include metabolic gases such as carbon dioxide (CO_2), hydrogen sulfide (H_2S), or ammonium based compounds known as total volatile basic nitrogen (TVB–N) (Zhai et al., 2017). TVB–N target metabolites are widely used to measure the freshness of meat and seafood. During food degradation, the changes in the pH and acidity suggest spoilage. Some of the researches suggested that anthocyanins extracted from red cabbage may be applied as active packaging material in food industry (Abedi-Firoozjah et al., 2022).

There are various studies on the freshness indicators used in food packaging, but there are very few studies on freshness indicators produced from natural, biodegradable, and edible materials (Abedi-Firoozjah et al., 2022). In this study, non-woven cotton pad obtained from natural cotton and extracts of purple cabbage achieved by water or a mixture of water and alcohol were used to obtain a freshness indicator. Since sensory pad materials are in contact with food in the process from packaging to consumption, these materials should be natural, edible, and biodegradable for the protection of human health, the continuation of ecological life, and food safety.

Red cabbage extracts prepared with two different solvents were used to prepare pH sensitive sensory pad for detection of chicken meat spoilage in the food package. The purple coloured pH sensitive sensory pad that is natural, biodegradable, and edible was put inside a chicken meat package to observe chicken meat spoilage. As a result, the colour of the sensory pad changed after the chicken meat had been spoiled with an accompanying pH change.

2. MATERIALS AND METHODS

2.1. Preparation of sensory pad

Red cabbage extract was achieved by solid–liquid extraction (leaching). Red cabbage leaves were cut into small pieces using a blender. Distilled water was used as solvent for the extraction of anthocyanins from red cabbage, because it is economical and convenient to use. Since the solvent was water, the temperature of the solvent was applied as 90 °C. The blended red cabbage was weighed as 100 g and placed in an 800 mL volume glass beaker containing 400 mL of preheated distilled water. The extraction mixture was stirred for 30 min with 300 r.p.m. stirring

rate under controlled heating conditions at 90 °C. After 30 min of the extraction process, the mixture was filtered with a filter paper (Whatman no. 1), and 5 mL purple extract (16 g L^{-1}) was taken to be dropped onto the cotton pad. The wet cotton pad was dried in an oven at 105 °C for 15 min, then it was sterilised for 15 min at 120 °C.

When the ethanol–water mixture (1:1 by volume) was used as solvent, the temperature of the extraction process was set to 79 °C. The extraction process was carried out with 300 r.p.m. stirring rate and at 79 °C temperature for 30 min. The extract (20.8 g L^{-1}) was filtered with a filter paper, and 5 mL purple extract was taken to be dropped onto the cotton pad. The wet cotton pad was dried in an oven at 105 °C for 15 min, then it was sterilised for 15 min at 120 °C.

2.2. Thermal characterisation of sensory pad

Thermal stability studies of cotton pad, red cabbage extract solution, and red cabbage extract solution on cotton pad (RCE–W) were applied with a thermal gravimetry-differential thermal analysis (TG–DTA, Shimadzu DTG–60) device between 30 and 120 °C temperatures with a heating rate of 10 °C·min^{-1} under air atmosphere.

2.3. Fourier transform infrared (FTIR) analysis

Fourier transform infrared (FTIR) spectrum analysis of cotton pad, RCE–W, and RCE–WE sensory pads was applied to investigate chemical structures with a JASCO FT/IR–4100 device in the range of 4,000–400 cm^{-1}.

2.4. Microscope analysis

Light microscope images of the cotton pad, RCE–W, and RCE–WE sensory pads were obtained with a light microscope (Ningbo Teaching Instrument Ltd. Co., China).

2.5. Meat pH and colour changes of the sensory pad

Initially and in the further process, pH and colour values (L^*, a^*, and b^*) were measured before packaging and after packaging with 3 days periods. Colour measurements were performed on the sensory pad surface at 10 different locations for each sample; mean values of L^*, a^*, and b^* from the samples were calculated. Colour values were measured with a Chroma Meter (Konica Minolta, CR–400/CR–410).

These values were used to calculate C^* (chroma), h^* (hue angle), and ΔE^* (total colour difference) using Equations (1), (2), (3), and (4) below (Freitas et al., 2020).

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \tag{1}$$

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{2}$$

$$\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{3}$$

$$\Delta L^* = L^* - L_0^*;\ \Delta a^* = a^* - a_0^*;\ \Delta b^* = b^* - b_0^* \tag{4}$$

pH values of the chicken meat was measured with a litmus paper (OF Universal test paper) *via* sticking the paper on the chicken meat surface and comparing the colour change of the litmus paper with the pH colour scale.

2.6. Microbiological studies

Chicken thighs were used in the study. Mechanically separated chicken thigh samples obtained from a commercial chicken meat processing enterprise were brought to the laboratory under +4 ± 1 °C cold environmental conditions. Each thigh sample was kept in 500 g packages on the sensor pads at +4 ± 1 °C in the refrigerator.

2.6.1. Experimental plan. Analyses were made at "0", "4", and "7" days using samples stored at +4 ± 1 °C. The experiment was carried out in three replications.

2.6.2. Preparation of chicken thighs for microbiological analysis. 10 g of each pack of chicken thighs were weighed into sterile plastic bags under aseptic conditions. After adding 90 ml of 0.1% sterile peptone water on it, it was homogenised in a mixer (vortex) for 2–3 min. Thus, a 10^{-1} dilution of the sample was prepared. From this dilution, other decimal dilutions up to 10^{-7} were prepared and planted (Harrigan, 1998). Total aerobic mesophilic bacteria (TAMB), total psychrotrophic aerobic bacteria (TPAB), coliform bacteria, *Staphylococcus* spp., *Enterococcus* spp., and yeasts and moulds were counted in chicken thighs.

2.6.3. Total mesophilic aerobic bacteria (TMAB) and psychrotrophic aerobic bacteria (TPAB) counts. After planting on plates containing Plate Count Agar (PCA) from the dilutions prepared, the Petri dishes were incubated at 30 ± 1 °C for 48 h and for 10 days at 4–5 °C for the count of TMAB and TPAB, respectively (Maturin and Peeler, 1998).

2.6.4.* Coliform *count. Violet Red Bile (VRB) agar was used in the enumeration of coliform group bacteria. 48 h of incubation at 37 °C was applied and the resulting typical colonies were counted (Gürgün and Halkman, 1990).

2.6.5.* Staphylococcus *spp. count. Mannitol Salt Agar (MSA) was used in the enumeration of staphylococci. 36–48 h of incubation at 37 °C was applied and the resulting typical colonies were counted (TOKIB, 1983).

2.6.6.* Enterococcus *spp. count. In Slanetz–Bartley Medium, 24–48 h incubation was performed at 44 °C, and then *Enterococcus* spp. colonies were counted (Halkman, 2005).

2.6.7. Yeasts and moulds count. Potato Dextrose Agar (PDA) containing 10% of tartaric acid was used for the determination of yeast and mould counts. After planting from the prepared dilutions, the Petri dishes were incubated at room temperature (22–25 °C) for 5 days (Harrigan, 1998).

3. RESULTS AND DISCUSSION

3.1. Thermal stability studies

It is important that the sensory pads are sterile during packaging processes. TG–DTA analysis was applied to see their stability against heat treatments. The TG–DTA curves of red cabbage extract solution containing cotton pads and cotton pads are shown in Fig. 1.

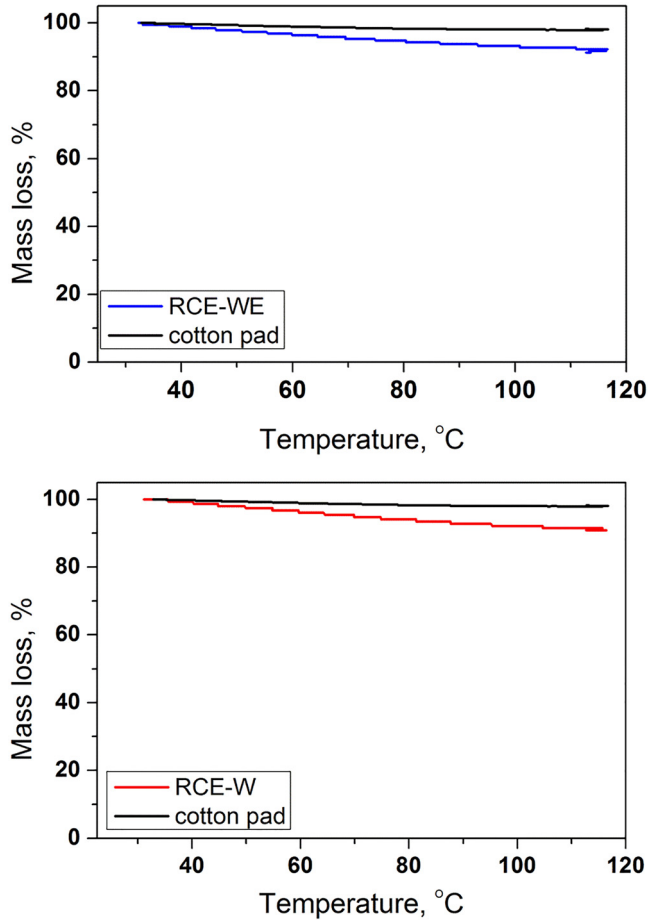

Fig. 1. TGA vs. temperature diagrams of cotton pads; RCE–W: smart sensory pad with red cabbage extract prepared using water as solvent; RCE–WE: with extract prepared using water-ethanol mixture (1:1 v/v)

There was a decrease in mass of around 10% for RCE–WE and RCE–W, while it is about 2.1% for cotton pad. After adding red cabbage extract, mass loss% increased to 10%. This loss corresponded to the evaporation to the bound water and low molecular weight compounds in the sensory pads. Red cabbage extract addition did not affect the thermal stability of the cotton pad, which exhibited stability and thermal resistance for smart food packaging.

3.2. Fourier transform infrared spectroscopy (FTIR) results

Fourier transform infrared spectroscopy (FTIR) analysis was applied for identification of the chemical bonds present in cotton pad, RCE–W and RCE–WE sensory pads (Fig. 2).

Ravanfar et al. (2018) declared in their study that the extract obtained using water solvent had the highest sugar content, while the extract obtained with the ethanol:water mixture solvent

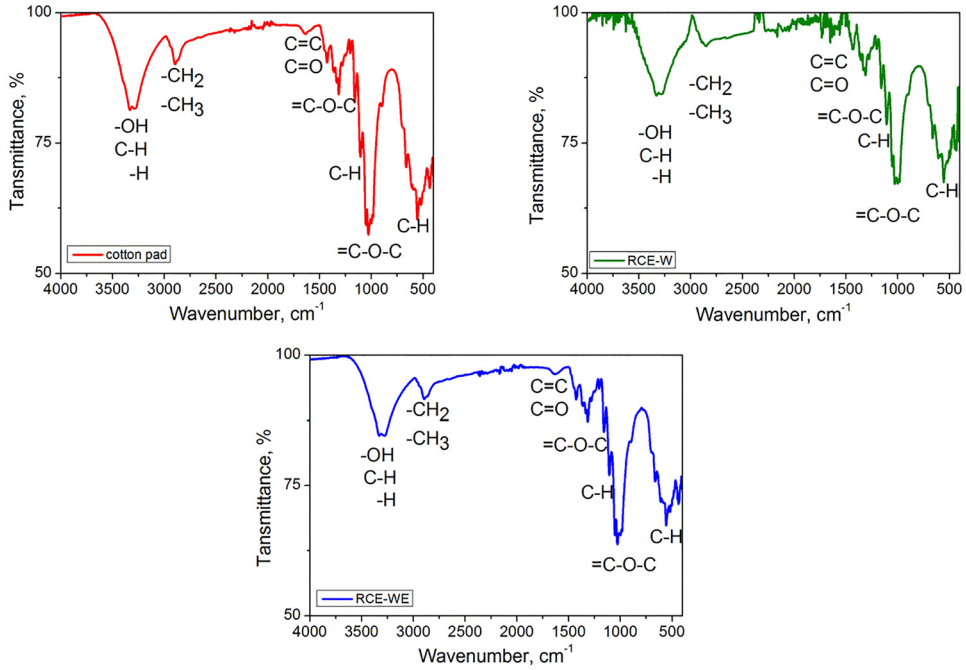

Fig. 2. FTIR spectrums of cotton pad, RCE–WE, and RCE–W

consisted of high amounts of phenolic components. Also, it has been reported that these phenolic components contained high amounts of anthocyanins. The FTIR spectras were very similar with only minor differences due to the similar chemical bonds of natural pigments and cellulosic structure of the natural cotton pad. The band at 3,000–3,500 cm^{-1} can be attributed to stretching of the –OH bond in the moisture residue (according to TGA results), cellulose molecules, and anthocyanins. Krysa et al. (2022) reported that the bands around 3,600–3,200 cm^{-1} are related to the H−bonds, and around 3,000–2,800 cm^{-1} to C–H groups in anthocyanines. The stretching vibration peaks about 2,928 cm^{-1} and 2,855 cm^{-1} indicated the presence of methyl group ($–CH_3$) and methylene group ($–CH_2$) belonging to aromatic compounds of flavonoids. Flavonoids are considered polyphenolic compounds. According to their skeletal structure, they can be typed such as flavone, flavonol, flavonone, biflavonoid, and chalcone. In 1,750 cm^{-1} to 1,500 cm^{-1} region, the vibrations belong to the C = C, and C = O groups of anthocyanins in the red cabbage (Sousa et al., 2016). The peaks about 1,516 cm^{-1} and 1,261 cm^{-1} are attributed to skeletal stretching vibration of the aromatic rings and = C – O – C group of flavonoid compounds (Sakulnarmrat et al., 2020). The peak at 1,115 cm^{-1} is attributed to C–H bonds in the anthocyanin rings (Krysa et al., 2022).

3.3. Microscopy studies

Microscopy study was applied using a light microscope with 600× magnification (Fig. 3).

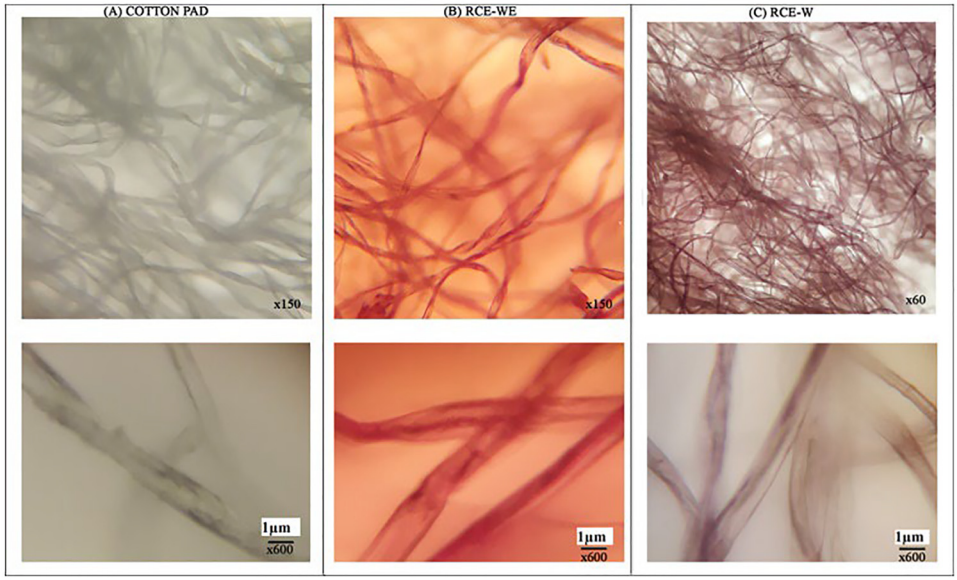

Fig. 3. Light microscope images of (A) Cotton pad, (B) RCE–WE, and (C) RCE–W

Microscopic images showed that the red cabbage pigments were absorbed into the cotton pad fibres with a uniform distribution. On the other hand, extracted pigments with different solvents exhibited different colours.

3.4. Colour change of sensory pad through storage time

The colour change of sensory pad was measured with a chroma meter before the storage and after the spoilage of chicken meat.

L^* (0 = darkness, 100 = lightness), a^* (+60 = red, −60 = green), and b^* (+60 = yellow, −60 = blue) values (Hamada et al., 1988) due to pH change are exhibited in Table 1. The measurements were performed with ten replications, and mean values were presented.

Schubring (2002) suggested that ΔE^* values higher than 3 are classified as "very pronounced differences to the human eye". Higher ΔE values mean that the RCE sensory pads have a good

Table 1. Colour parameters of the prepared sensory pads (RCE–W and RCE–WE)

Red cabbage extract prepared with water (RCE–W)				
Time (day)	L^*	a^*	b^*	pH
0	64.06 ± 1.49	19.63 ± 0.84	6.55 ± 0.85	6
7	62.22 ± 1.91	0.64 ± 1.50	1.56 ± 1.02	8
Red cabbage extract prepared with water and ethanol (1:1 v/v) (RCE–WE)				
Time (day)	L^*	a^*	b^*	pH
0	56.92 ± 1.86	26.00 ± 1.26	−7.94 ± 0.74	6
7	75.72 ± 2.26	−1.02 ± 0.98	18.89 ± 1.23	8

colour variation, due to pH alterations. In this study, ΔE^* values were calculated for sensory pad RCE–W and RCE–WE as 19.72 and 42.47, respectively. The calculated colour intensity (chroma, C^*) and Hue angle (h^*) values are also presented in Table 2. The Hue angle was calculated using Equation (2), where 0° is red, 90° is yellow, 180° is green, and 270° is blue.

While the colour intensity values decreased for both sensory pads, the Hue angle changes exhibited the colour variations. The colour change of the RCE–WE sensory pad was observed in the 0–90° region, which indicated a yellow ratio increase. RCE–W sensory pad colour changed in the negative region between 16.98° and 86.91° Hue angles, which indicated an increase of blue colour ratio. pH value of the fresh chicken thigh was measured as 6. When the chicken meat spoiled with a bad odour, the pH value was 8, and the colour of the sensory pad changed to blue as shown in Fig. 4.

The extract concentrations were 16 g L^{-1} and 20.8 g L^{-1} for RCW–W and RCW–WE, respectively. The extraction yield of water:ethanol mixed solvent was higher than of the water solvent. Since anthocyanins have hydrocarbons that are resistant to water and polyphenol compounds that are highly soluble in water and polar solvents, the solvent that is a mixture of water and ethanol in anthocyanin extraction provides high efficiency. This solvent mixture is not only able to achieve high extraction efficiency, but is also safe for human health and inexpensive. Ravanfar et al. (2018) extracted the anthocyanins from red cabbage using water, water:ethanol (1:1 by vol.), and ethanol solvents, separately. They found that the fraction obtained through the fractionation with ethanol/water (1:1) showed the highest antioxidant activity, anthocyanin content, and phenolic compounds. Thus, the extraction yield with water: ethanol solvent was higher and the color change of RCW–WE was more noticeable than of RCW–W.

3.5. Microbiological flora studies

The data of the microbiological flora are presented in Table 3.

According to the Turkish Food Codex Raw Poultry Meat and Prepared Poultry Meat Mixtures, the maximum value determined for the number of TMAB for raw poultry meats is 5.0×10^6 CFU g^{-1} in 2 out of every 5 samples (Anonymous, 2006). Sağun et al. (1996), in their study on the hygienic quality of chicken thighs and chicken breasts, found the general colony number in thighs as 1.4×10^6 CFU g^{-1}. In our study, the 0th day value was found to be 5.30 log CFU g^{-1}, the 4th and 7th day values were 7.60 log CFU g^{-1} and 7.90 log CFU g^{-1}, respectively. When the results converted to the relevant unit were used to compare the results in the

Table 2. Calculated C^* and h^* values of RCE–WE and RCE–W sensory pads when the pH value of the chicken meat changed from pH 6 to pH 8

	C^*		h^*	
	pH 6	pH 8	pH 6	pH 8
RCE–WE	20.69	1.69	18.45	67.69
RCE–W	27.19	18.92	−16.98	−86.91

RCE–W: sensory pad with red cabbage extract prepared using water as solvent; RCE–WE: with extract prepared using water-ethanol mixture (1:1 v/v).

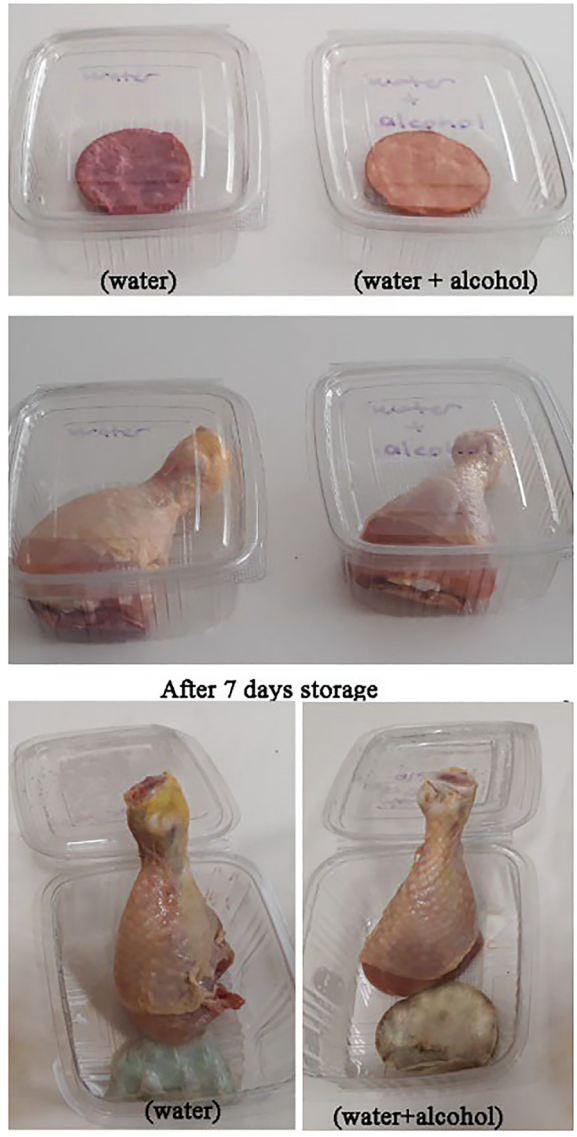

Fig. 4. Colour change of sensory pad after storage and spoilage of chicken thigh

literature, TMAB number of thigh samples were found to be low on day 0 and similar to the results of other researchers on day 4 and 7.

The TPAB content of chicken breasts and thighs is important, since psychrophilic and psychrotrophic bacteria are the most important bacteria group for cold stored foods. It is the number of psychrophilic bacteria that is accepted as an indicator of spoilage in the cold storage of foods. Efe and Gümüşsoy (2005) and Yıldırım et al. (2015) found TPAB in thigh samples

Table 3. Number of indicator microorganisms causing spoilage of chicken thigh samples (log CFU g^{-1})

Microorganism	0th day	4th day	7th day
Total Aerobic Mesophilic Bacteria (TAMB)	5.30	7.60	7.90
Total Psychrotrophic Aerobic Bacteria (TPAB)	4.31	6.66	6.86
Coliforms	3.92	7.30	7.55
Staphylococcus spp.	2.00	2.90	4.74
Enterococcus spp.	1.18	3.30	3.79
Yeasts and moulds	<2	<2	<2

between 2.9×10^4–1.79×10^7 CFU g^{-1}. According to this study, when the unit of the results were converted to CFU g^{-1} unit to be able to be compared to results in the literature, TPAB results were similar to the results of other researchers.

Since coliform bacteria are common in both the intestine and nature, they are considered as an indicator of sanitation in the food industry (Yıldırım et al., 2015). However, the coliform group bacteria was 3.92 log CFU g^{-1} on the 0th day, it was 7.30 log CFU g^{-1} and 7.55 log CFU g^{-1} on the 4th and 7th day, respectively. Anar et al. (1992) reported that they found a coliform group microorganism at the level of 6.1×10^1 CFU g^{-1}–3.0×10^5 CFU g^{-1} on chicken thighs. Our 0th day study result was similar to findings of Anar et al. (1992), and after storage, on the 4th and 7th day, a deterioration could be observed in the microbial state of the samples.

S. aureus is one of the pathogenic bacteria of great importance in meat and meat products. *S. aureus* content of chicken thighs causes food poisoning by secreting high temperature resistant protein enterotoxins that cause disease in humans. It is stated in the Pathogen Limits section of TGK Regulation on Microbiological Criteria (TGK, 2011) that the limit of coagulase positive *S. aureus* should be 10^3 CFU g^{-1}. In this study, the number of *Staphylococcus* spp. was found to be 2.00 log CFU g^{-1} on the 0th day, 2.90 log CFU g^{-1} on the 4th day, and 4.74 log CFU g^{-1} on the 7th day. Therefore, the load on the 7th day was above the limit, which indicated deterioration and disease-causing characteristics. Also, Yıldırım et al. (2015) determined the number of *Staphylococcus* spp. as $<10^2$–2.02×10^5 CFU g^{-1} when investigating the microbiological properties of chicken thighs offered for sale in their study. Those results were similar to the results obtained in this study.

Enterococcus spp. is widely found in nature. Enterococci can be used as indicator microorganisms to show faecal contamination in determining the microbiological quality of foods. *Enterococcus* spp. number for chicken thigh was reported as 2.49 log CFU g^{-1} by Atlan and İşleyici (2012). Capita et al. (2001) determined *Enterococcus* spp. number in all chicken samples as 2.72 log CFU g^{-1}. When the findings were compared with the results of our study, it was seen that the 0th day value was lower than these values, and the 4th and 7th day values were higher than these values. In this case, microbiological quality was low and indicated deterioration.

The number of yeasts and moulds is considered as an indicator of deterioration in food products. The maximum allowed value for poultry meat mixtures according to the Turkish Food Codex Regulation Communique on Raw Poultry Meat and Prepared Poultry Meat Mixtures is 1.0×10^4 CFU g^{-1} in 3 out of 5 samples (Anonymous, 2006). Atlan and İşleyici (2012) reported the number of yeasts and moulds in chicken thighs to be 3.75 log CFU g^{-1} in their study. The

yeasts and moulds numbers in this study was found to be lower than that. Accordingly, the results obtained on the 0th day was <2 log CFU g^{-1}, on the 4th day was <2 log CFU g^{-1}, and on the 7th day was <2 log CFU g^{-1} in our study. In this case, yeasts and moulds were not the reason of deterioration of chicken thigh.

4. CONCLUSIONS

In this study, after storing chicken meat for 7 days, the colour change of the RCE–WE sensory pad was more pronounced than of the RCE–W sensory pad. The developed pH sensitive sensory pads are non-toxic because of being prepared from red cabbage and cotton. The developed pH sensory pads present a simple, low cost, visual method based on colour changes to detect the freshness of chicken thigh.

Conflict of interest: The authors declared that they have no conflict of interest.

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