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## Full Research Paper

# Development of Mozzarella cheese freshness indicating film by embedding purple carrot extract in gelatin and Persian gum matrix

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**Abstract**

This study aimed to evaluate the effects of intelligent pH-sensitive composite film based on gelatin and Persian gum incorporated with purple carrot extract (PCE) on the freshness of wrapped mozzarella cheese. In this regard, the color, pH, yeast and mold count of control and treatments wrapped with intelligent pH-sensitive composite film during 60 days were evaluated. The results showed that the pH significantly reduced in wrapped cheese with and without PCE (control) samples during storage. However, this reduction was more pronounced in the control sample (P<0.05). Additionally, the application of composite film on cheese affected the color during storage. It was observed that L* and *a** values of the composite film-wrapped cheese were significantly higher than the control sample, but the *b** values were significantly lower than the control sample. Moreover, poor microbial growth (yeasts and mold) was observed in cheese samples wrapped by composite film with purple carrot compared to the control. Also, the pH of the composite film with extract significantly decreased from 6.33 to 4.85 during storage (P < 0.05), which showed the changes of color from purple to pink. After 40 days, the color changed to pink, indicating the end of the cheese storage. Therefore, it was concluded that the pH-sensitive film, while being an effective method to improve the shelf life of mozzarella cheese, can also use as an indicator for freshness.

**Keywords**: Gelatin; Mozzarella cheese; Persian gum, pH-sensitive film; Purple carrot extract.

## Introduction

Edible coatings and films are thin layers of various natural materials, such as polysaccharides, proteins, and lipids which coat the surface of fruits and vegetables (Al-Hassan & Norziah, 2012). They act as an excellent physical barrier to water and simple gases such as $CO_2$ and $O_2$, leading to reduction of deterioration and oxidation, and moisture loss (De Pilli, 2020; Suhag et al., 2020). Polysaccharide and protein coatings and films can effectively extend the shelf life of different foods due to their hydrophilic nature. Also, it has been reported that coating\film made of lipid components such as paraffin and wax can significantly reduce water loss from fruits and vegetables because they provide a more effective barrier toward water evaporation

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(Jafarizadeh Malmiri et al., 2012). Therefore, development of coatings and films from both hydrophobic and hydrophilic biomolecules with suitable nutritional and sensorial characteristics could be a useful packaging technique to preserve perishable food products and enhance their shelf life (Jafarizadeh et al., 2011). The different features of coatings materials like type, amount, density, viscosity, and surface tension as well as the techniques of coatings applications, directly influence edible coatings performances (Zhong et al., 2014).

Persian gum is one of the main carbohydrates recommended for food applications in Iran. Recently some researchers reported that this gum had good properties in the production of edible films (Pak et al., 2020; Tabatabaei et al., 2022).

Gelatin is one of the main proteins used in the production of film and coating all around the world. Application of these ingredients in the composite films can improve the physicochemical, mechanical, and structural properties (Akrami-Hasan-Kohal et al., 2020). The intelligent coating is a type of system applied for packaging of food. In this system, some changes during the shelf life was monitored. Sensors, data carriers, and indicators are important parts of this method for evaluation the quality of food products. Usually, this method is based on data collection and evaluation of some food parameters including pathogens, carbon dioxide, oxygen, temperature, freshness, leakages, pH-level (Azman et al., 2022; Schaefer & Cheung, 2018).

PCE (purple carrot extract) is an ingredient with pH-sensitive properties. The color of this extract is depended on the pH of the solution and can be changed from purple to pink at different pHs. Purple carrots (*Daucus carota subsp. Sativus var. Atrorubens Alef*) grown in the Middle East and Europe. The acylated anthocyanins in purple carrots are a natural pigment suitable for coloring foods (Arab et al., 2023). One of the main ingredients in intelligent films is pH-sensitive materials. Therefore, this extract can act as a part of this film.

Cheeses are a valuable source of nutrients and probiotics which have been an important part of dairy consumption around the world. Soft cheese products are prone to microbial spoilage by a wide range of microorganisms, resulting in short shelf life. Fresh Mozzarella cheese is a white cheese of the pasta filata family of Southern Italy origin, which is cut and produced in various shapes. Owing to high moisture content, fresh Mozzarella considered highly perishable product with a short shelf life of approximately 5 to 7 d. Moreover, the growth of spoilage microorganisms such as Pseudomonas *spp* and coliforms is another main factor limiting Mozzarella cheese shelf life (Altieri et al., 2005). Therefore, several attempts have been conducted to introduce effective techniques that can extend the shelf life of this popular cheese. In this regard, the application of edible coatings and films on cheeses could be a promising strategy for cheese preservation and extension of their shelf life (Costa et al., 2018). However, limited studies and few commercial examples have been found regarding various coating materials and also technologies applied to cheese products (O'Callaghan & Kerry, 2016). Thus, the objective of the present study is to evaluate the effects of an intelligent pH-sensitive composite film based on gelatin and Persian gum incorporating PCE on the freshness of mozzarella cheese.

## Materials and methods

Bovine gelatin (bloom 200) was purchased from Gelatin Halal Co. (GHC, Tehran, Iran) and Persian gum was obtained from a local market in Shiraz (Fars Province, Iran). Glycerol as the plasticizer was prepared from Emboy Kimya (Turkey). Purple carrots were purchased from a local market in Bandar Abbas (Iran). Ethanol, HCL, and NaOH were from Merck CO. (Germany) and YGC was prepared from Hi-Media, (India).

### Extract preparation

The ripe purple carrot was used for anthocyanin extraction according to the method of Wang et al. (2021). First, 100 g of carrot cuts was mixed with 500 mL of 80% ethanol solution containing 1% HCL (12 M) for 24 h at 4°C. Then the mixture was centrifuged at 8000 g for 15 min at 4°C to obtain anthocyanin-rich extract.

**Film preparation**

Gelatin, Persian gum and glycerol solutions were obtained by dissolving each of them in 100 cc of a solution containing 45 cc of PCE and 55 cc of distilled water with ratios of 5, 5 and 3% w/v respectively. The prepared solutions were mixed by a magnetic stirrer for 6 h. Films were prepared by pouring 20 ml of the mixture into the Petri dishes. In order to make a thin layer, the solutions were spread on the surface by a glass rod with a 10-centimeter diameter and then put aside to dry at ambient temperature for 48 h and 24 h in the desiccator (Arab et al., 2023).

**Determination of pH-sensitivity of film**

In order to investigate color changes, the film was flooded with an aqueous media with different pHs from 2 to 12 for about 15 min. The color change was recorded by Minolta colorimeter (CR-20, Konica Minolta, Inc., Tokyo, Japan). The color parameters of cheese samples in terms of lightness (*L**), redness (*a**), and yellowness (*b**) were determined, then the color difference ($\Delta E$) for the film at different pHs compared to a constant pH (pH =2) was calculated through the following equation:

$$\Delta E = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2} \qquad (1)$$

L*, a* and b*(Color indexes of the film at different pH)

$L_0$, $a_0$ and $b_0$ (Color indexes of the film at pH equal to 2)

**Cheese preparation**

Finger mozzarella cheese was prepared by adding mesophilic-thermophilic starter culture (Chr-Hansen Company) to the high-fat milk which was heated at 85°C for 15 s. After cheese milk coagulation, whey was separated from the freshly formed curd in a special cheese vat to obtain curd with 48% dry matter. The dry curd was maintained until the desirable pH of 5.2 was reached.

Then the curd was transferred to the cooker stretcher at 75°C equipped with a direct heating system and two spirals with reverse rotational directions. The other ingredients were added to the curd in this stage. The subsequent stage was transferring curd to Mulder for 10 min. Then, the curd was immersed in cold water at 1- 3°C to stabilize the structure. Finally, mozzarella cheese was packed and kept in cold storage (Bermúdez-Aguirre & Barbosa-Cánovas, 2012).

**Film application**

Two stripes of the prepared edible film (9.5 x 7.5 cm) were heat sealed on three sides to form the pouch, cheese sample was then placed into the pouch followed by air removal under vacuum. The automatic machine equipped with a vacuum pump (60 cm/Hg- 0.80 bar/11.6 PSI) was used to completely seal the pouch. PE with the mentioned dimensions was also used as secondary packaging to cover the mozzarella cheese samples. All cheese wrapping samples were stored at 4°C.

**Color of cheese**

The color parameters of cheese samples in terms of lightness (*L**), redness (*a**), and yellowness (*b**) were determined using a Hunter colorimeter (Model CR- 300, Minolta Camera Co, Tokyo, Japan) as described by Dai et al. (2018).

**pH of cheese samples**

The pH of cheese samples was measured using a digital pH meter and titration method according to Iran's national standard No. 2852 (Öztürk & Güncü, 2021).

**pH of film samples**

The film samples (1 g) were mixed with 9 mL of deionized water for 1 min. pH values were measured at room temperature using a digital pH meter by direct immersing the electrode into the mixture.

**Microbiological analysis**

Determinations of yeast and mold counts were performed by inoculating the required dilutions in YGC (Hi-Media, India) and incubated at 28°C for 72-120 h, to form visible colonies. Results were interpreted as the presence or absence of yeast and molds in 1g of the sample (Tirloni et al., 2019).

**Statistical analysis**

All experiments were conducted in triplicates. The design of the completely randomized analysis of variance (ANOVA) procedure was conducted using SAS Statistical Software (Version 9.1 SAS Institute Inc., 2000; Cary, NC, US). Duncan multiple range tests were performed to compare mean values at ($p$<0.05).

## Results and discussion

**pH sensitivity of film**

The pH response and ΔE of PCE-loaded film are shown in Fig. 1 and Table 1 respectively. Film samples were shown pH- sensitivity and notable color changes as affected by different buffer solutions..

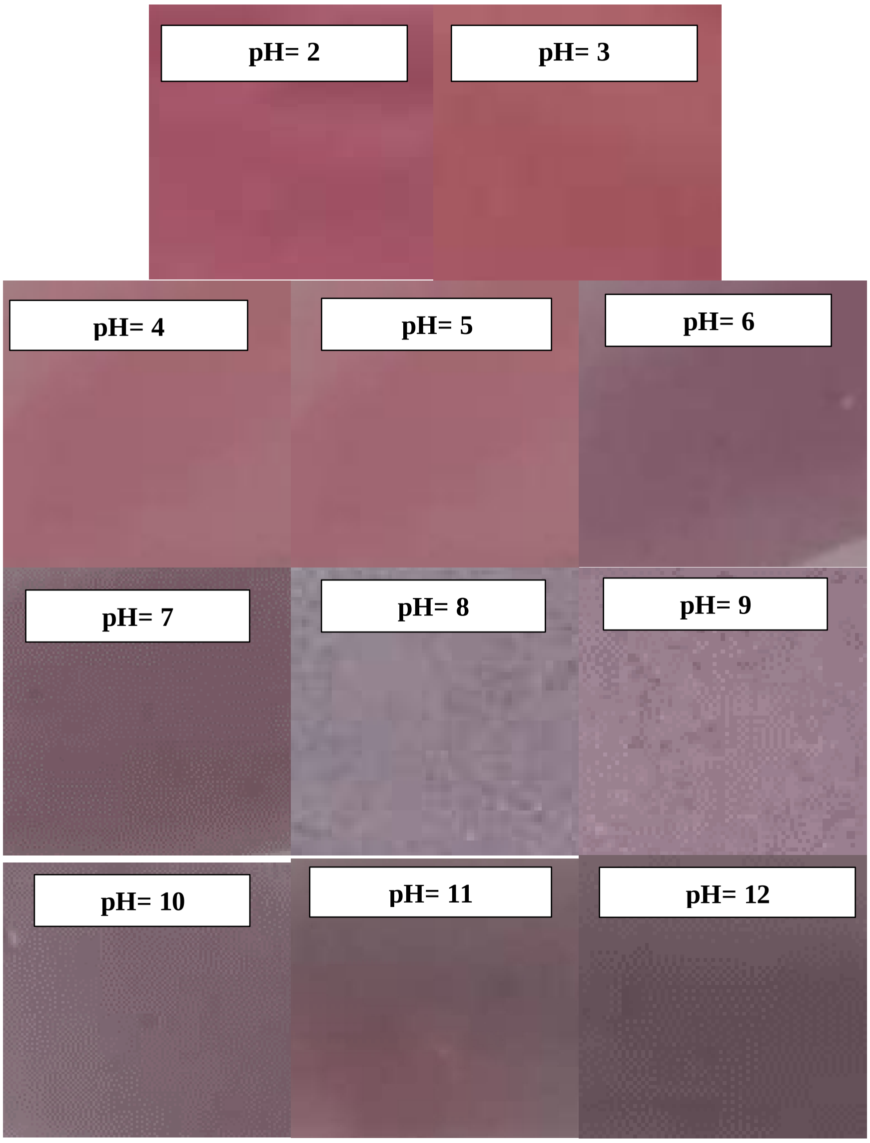


**Fig. 1. Color of composite film based on gelatin and Persian gum incorporated with PCE in different pHs.**

**Table 1- Color difference (ΔE) of composite film based on gelatin and Persian gum incorporated with PCE in different pHs.**

| pH | L* | a* | b* | ΔE |
|---|---|---|---|---|
| 2 | $46 \pm 1^a$ | $35 \pm 1^a$ | $11 \pm 3^a$ | - |
| 3 | $47 \pm 1^a$ | $33 \pm 1^b$ | $5 \pm 1^b$ | $6.403^a$ |
| 4 | $50 \pm 2^b$ | $25 \pm 2^c$ | $4 \pm 1^b$ | $8.602^b$ |
| 5 | $60 \pm 2^c$ | $21 \pm 1^d$ | $3 \pm 1^b$ | $10.817^c$ |
| 6 | $43 \pm 2^d$ | $19 \pm 1^e$ | $2 \pm 1^b$ | $17.146^d$ |
| 7 | $40 \pm 2^e$ | $18 \pm 1^e$ | $0 \pm 1^c$ | $3.742^e$ |
| 8 | $55 \pm 2^f$ | $15 \pm 1^f$ | $-1 \pm 1^d$ | $15.330^f$ |
| 9 | $57 \pm 1^f$ | $13 \pm 1^g$ | $-1.3 \pm 0.3^g$ | $2.844^g$ |
| 10 | $45 \pm 2^d$ | $12 \pm 1^h$ | $-1.5 \pm 0.2^g$ | $12.043^h$ |
| 11 | $35 \pm 2^g$ | $11 \pm 1^h$ | $-1.8 \pm 0.2^g$ | $10.054^i$ |
| 12 | $38 \pm 2^h$ | $10 \pm 1^h$ | $-2.0 \pm 1.0^h$ | $3.169^j$ |

Different letters in each column indicate significant differences ($P < 0.05$).

By increasing pH values, the color of the film turned from pink to blue/purple. The structural changes of anthocyanin at different pHs causing the color changes. Anthocyanins exist basically in cationic form (red or pink flavylium cation) at pH 2– 3. The purple color observed at high pH (pH 4– 6) due to the generation of carbinol pseudo-base and the blue-colored observed at pH> 7 due to the formation of quinoidal bases (Koosha & Hamedi, 2019). Other researches also showed the pH sensitivity of biopolymer-based films incorporated with extracts of purple potato, red cabbage, blackberry pomace, and purple and black eggplant (Yong, et al., 2019). Changes in the color of the sample were validated by measuring the ΔE of samples. The difference ($p< 0.05$) in the amount of ΔE indicates the color change of the film at different pH. The results proved that PCE-loaded film could act as a pH indicator.

**Color of cheese**

The observed values for *L**, *a**, and *b** are represented in Fig. 2, 3, and 4 respectively. Color is considered one of the critical characteristics of cheese, which affects consumer acceptability and taste perception. Also, the color could be an excellent indicator for consumers to evaluate the freshness and quality of various products (Dong, et al., 2020). At the beginning of the storage, *L** values for all treatments were between 59- 70 and 76- 82 for the control. This value declined in all samples after 60 days of storage, which could be attributed to microorganism growth and lipid oxidation in products (Cerqueira, et al., 2009; Huang et al., 2018).

Similar results were reported regarding the significant reduction of *L** value during 10 days of storage in cheese coated with sodium alginate, the sample coated with sodium alginate containing 1% *Pimpinella Saxifraga* essential oil, and the control sample (Ksouda, et al., 2019). Mei et al. (2015) also reported that the growth of mold and yeast during 20 days of refrigeration storage on the cheese surface, caused *L** value reduction in uncoated and coated bod liong cheese samples (a type of semi-hard cheese) with chestnut starch-chitosan mixture and chestnut starch-chitosan enriched with pine fruit essential oil or *Cornus officinalis* fruit.

As illustrated in Fig. 3 and 4, the *a** values for the wrapped cheese with the composite film were significantly higher than the control, although the *b** values were lower in the control sample. Despite the protective effects of films in preventing the growth of microorganisms and the antioxidant properties of film, these samples had lower *L** and *b** values than the control sample, while the values of a* were higher compared to the control sample. Several studies proved that wrapping materials had major effects on the discoloration of food items during shelf life. Pena-Serna et al. (2016)

indicated that Mongolian cheeses coated with zein and zein-xanthan solutions were more subjected to color changes compared to uncoated samples. They stated that increasing *b** and *a** values and decreasing *L** values immediately after wrapping were related to the yellowish color of the wrapping solutions. Ramos et al. (2012) also reported higher color changes in cheese samples coated with whey-gum protein isolate. Similarly, previous research on coated Queso Blanco cheese (a type of fresh cheese with a soft texture) containing flax oil (QB-FO) with whey protein isolate (WPI) and also WPI containing oregano essential oil reported a significant reduction of *L** values during the storage period compared to the control sample. Moreover, they reported the *b** values of wrapped samples increased drastically during the storage and were significantly higher than QB-FO at the end of the storage period (Gurdian, et al., 2017). Thus, selecting the proper materials for coating and film preparation effectively improves the color of this well-known dairy product and hence directly affects consumer acceptance.

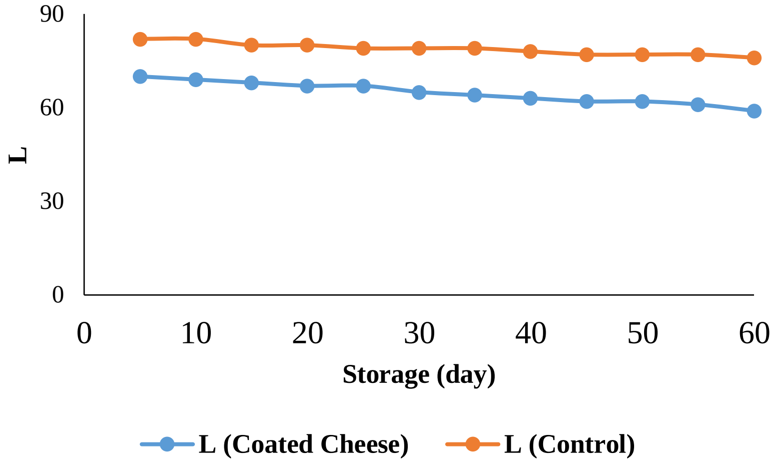


**Fig. 2. *L** values of mozzarella wrapped with the composite film based on gelatin and Persian gum incorporated with PCE, and control during 60 days of storage.**

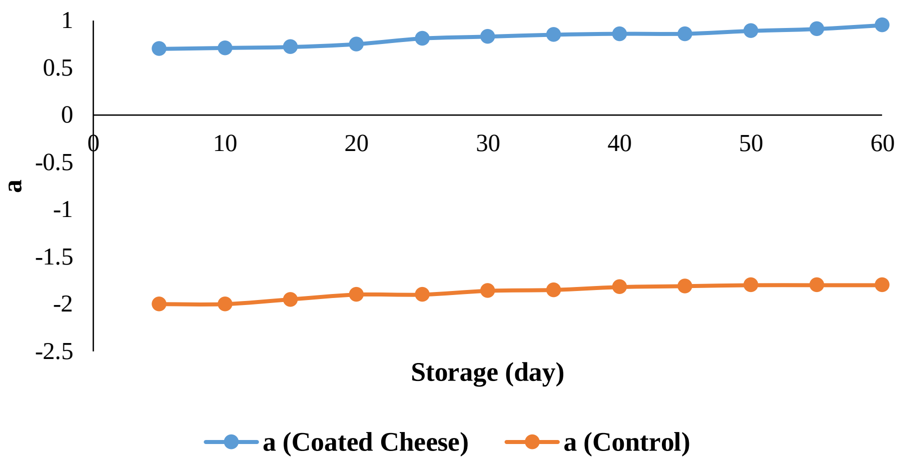


**Fig. 3. *a** values of mozzarella wrapped with the composite film based on gelatin and Persian gum incorporated with PCE, and control during 60 days of storage.**

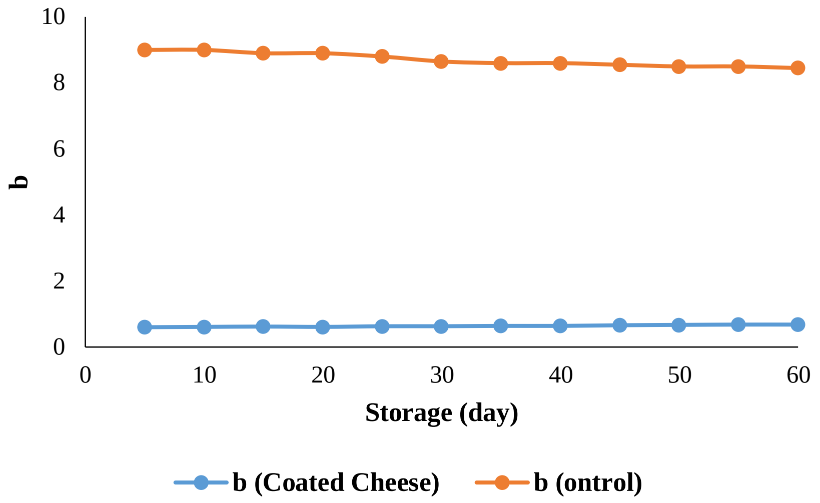


**Fig. 4.** ***b**** **values of mozzarella wrapped with the composite film based on gelatin and Persian gum incorporated with PCE, and control during 60 days of storage.**

**pH of cheese**

The pH values as an important quality parameter of cheese products were monitored during refrigerated storage. The results of many studies revealed that pH changes can directly affect the structural properties and chemical composition of cheese. The pH reduction throughout the storage significantly promotes mineral solubility and also changes casein micelles structure, leading to subsequence changes in the nature and intensity of protein interactions in cheeses (Pastorino et al., 2003). Moreover, Hayaloglu (2016) reported that the flavor and total microbial counts of cheeses are also drastically affected by the pH values. Thus pH can be a good indicator of various bacterial growth, including coliforms or pathogenic bacteria.

The pH values of wrapped cheese with the composite film and control cheese during 60 days in the refrigerator can be seen in Fig 5. The pH standard of mozzarella cheese is between 5.1 and 5.3. Regardless of the film types, the pH levels decreased in both cheese samples which was significant for the control sample ($P<0.05$). This reduction can be attributed to the conversion of lactose into lactic acid by increasing lactic acid bacteria activities such as *Lactococcus* and *Lactobacillus* in cheese during storage (Evert-Arriagada et al., 2018). The present results were in good agreement with previous reports by Ramos et al. (2012) of pH reduction in whey protein isolate coating of semi-hard cheese due to lactic acid production. Mahcene et al. (2021) also reported that microbial fermentation and peptides production caused a pH decrease in freshly coated homemade cheeses with sodium alginate containing essential oils. Similar changes in pH were found for ricotta cheese prepared by thermal coagulation method and treated with a chitosan-whey protein mixture during the first 6 days of storage. The production of amino acids and Free Fatty Acids during proteolysis and lipolysis were the main reason for this reduction. However, no significant differences were observed between the coated and control samples (Di Pierro et al., 2011). Moreover, Amjadi, et al. (2019) stated that the release of $CO_2$ as a result of the subsequent metabolism of lactate as well as the decarboxylation of amino acids led to pH reduction on the surface of the white cheese.

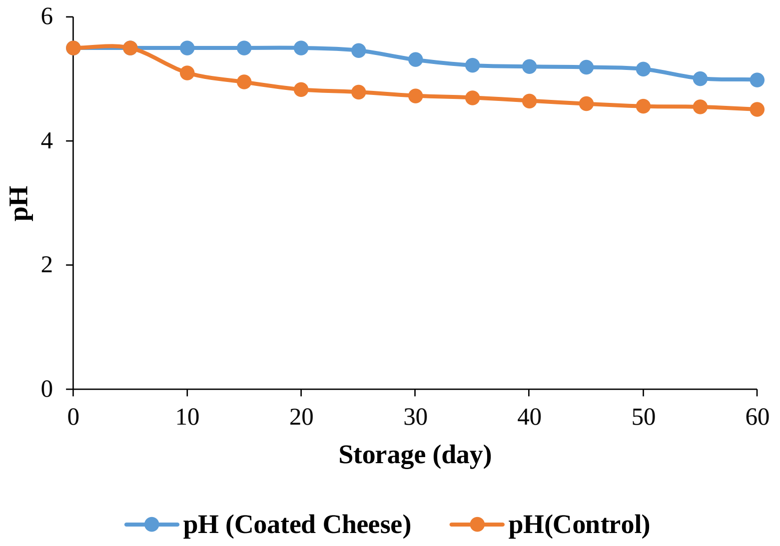


**Fig. 5. pH of mozzarella wrapped with the composite film based on gelatin and Persian gum incorporated with PCE, and control during 60 days of storage.**

**pH of the film**

Figure 6 shows the pH values for the composite edible film during 60 days of storage. The pH of the film significantly decreased from 6.33 to 4.85 during the storage period (P<0.05). As described previously in section 3.1, the pH reduction of the film could be related to producing free fatty acids and amino acids (Mahcene, et al., 2021; Pirsa, Karimi Sani, Pirouzifard, & Erfani, 2020). The color of films turned to pink at pH 5.

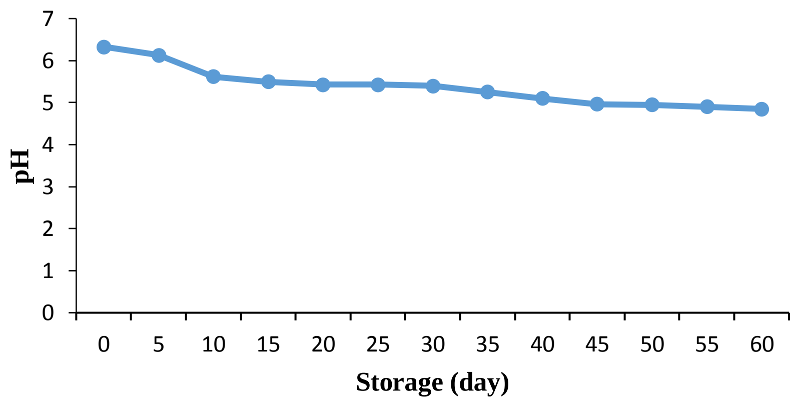


**Fig. 6. pH of the composite edible film based on gelatin, Persian gum incorporated with PCE during 60 days of storage.**

**Yeast and mold**

Yeasts and mold contaminations are common threats to dairy products which promote some defects such as off-odor and off-flavor (Trmčić, et al., 2016). In fact, suitable acidity conditions and high water activity of cheese surface made it a great medium for various microorganisms (Proulx, et al., 2017). Since yeasts are capable of growth even under anaerobic conditions, monitoring yeast existence in dairy products is very critical (Mileriene, et al., 2021). Microbial deterioration may occur during the manufacturing, storage, and even transportation of both raw milk and final cheese products (Ferrão, et al., 2016).Besides, the serious health issues of consuming spoiled cheese, the identification of microbial contamination in cheese products could be a major financial loss for dairy factories. Thus, any successful efforts to inhibit mold and yeast growth effectively enhance the handling, marketing, and shelf life of cheese and could be a great opportunity for exporting this dairy product (Trmčić, et al., 2016). The application of edible film or wrapping to cover chees surface is a desirable approach to extend the shelf life and protect the cheese from further microbial contaminations (Kumar, 2019). Table 2 shows mold and yeast count during 60 days of storage. Based on the results, the control was more exposed to microbial growth compared to the wrapped cheese with composite film. Moreover, the storage time had a significant effect on mold and yeast proliferation in the control sample ($P<0.05$). Although, mozzarella cheese wrapped with composite films was completely resistant to microbial contamination, and the total counts of yeast and mold were not changed during storage. Similarly, Youssef et al. (2016) reported that soft Egyptian cheese coated with chitosan and carboxymethyl cellulose had no mold contamination, while a significant proliferation of 2.06 log CFU/g was observed for the control sample. Several studies also confirmed the effectiveness of edible coating and film application in preventing microbial growth on cheeses (Ramos, et al., 2012; Resa et al., 2016).

**Table 2- Mold and yeast count (Log CFU/g) in mozzarella wrapped with the composite film based on gelatin and Persian gum incorporated with PCE and, control during 60 days of storage**

| Day | Wrapped Cheese | Control |
|---|---|---|
| **0** | <10 | <10 |
| **5** | <10 | <10 |
| **10** | <10 | 1 |
| **15** | <10 | 1.69 |
| **20** | <10 | 1.90 |
| **25** | <10 | 2.30 |
| **30** | <10 | 2.81 |
| **35** | <10 | 2.84 |
| **40** | <10 | 2.91 |
| **45** | <10 | 2.91 |
| **50** | <10 | 2.92 |
| **55** | <10 | 2.96 |
| **60** | <10 | 2.97 |

**Conclusion**

The effects of intelligent pH-sensitive composite film prepared by gelatin, Persian gum, and PCE on the properties of wrapped mozzarella cheese were studied. Results showed that the pH significantly reduced in wrapped cheese with and without PCE (control) samples during storage. However, this reduction was more pronounced in the control sample. Additionally, the application of composite film on cheese affected the cheese's color during its storage. It was observed that $a^*$ values of the composite film-wrapped cheese were significantly higher than the control sample. Moreover, poor microbial growth (yeasts and mold) was observed in wrapped cheese by film composite with purple carrot compared to the control. After 40 days, the color changed to red, which means the end of shelf life of cheese. Finally, the results showed that this system can be used as a good intelligent pH-sensitive film for food products.

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# ساخت فیلم نشان‌دهنده تازگی پنیر موزارلا با جاسازی عصاره هویج بنفش در شبکه ژلاتین و صمغ ایرانی

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## چکیده

مطالعه حاضر با هدف بررسی تأثیر استفاده از فیلم کامپوزیتی حساس به pH مبتنی بر ژلاتین و صمغ ایرانی همراه با عصاره هویج بنفش (PCE) بر تازگی پنیر موزارلا انجام شد. در این راستا، رنگ، pH و تعداد مخمر و کپک نمونه شاهد و نمونه بسته‌بندی شده با فیلم کامپوزیتی حساس به pH در مدت ۶۰ روز مورد ارزیابی قرار گرفت. نتایج حاضر نشان داد که pH در نمونه‌های پنیر بسته‌بندی شده با و بدون PCE (شاهد) در طول نگهداری کاهش معنی‌داری داشت، اما این کاهش در نمونه شاهد بیشتر بود ($p <$ ۰/۰۵) علاوه بر این، استفاده از فیلم کامپوزیت بر روی رنگ پنیر در طول نگهداری تأثیر می‌گذارد. مشاهده شد که مقادیر $L^*$ و $a^*$ پنیر بسته‌بندی شده با فیلم کامپوزیت به‌طور قابل توجه بالاتر از نمونه شاهد بود، اما مقادیر $b^*$ به‌طور قابل توجه کمتر از نمونه شاهد بود. علاوه بر این، در پنیر بسته‌بندی شده توسط کامپوزیت فیلم حاوی هویج بنفش نسبت به شاهد، رشد میکروبی ناچیزی (مخمرها و کپک) مشاهده شد. همچنین در طول نگهداری pH فیلم کامپوزیت حاوی عصاره به‌طور قابل توجهی از ۶/۳۳ به ۴/۸۵ کاهش یافت ($p <$ ۰/۰۵) که منجر به تغییر رنگ از بنفش به صورتی شد. پس از ۴۰ روز، رنگ به صورتی تغییر کرد که نشان‌دهنده پایان نگهداری پنیر است. بنابراین نتیجه‌گیری شد که فیلم حساس به pH در عین حال که یک روش موثر برای بهبود ماندگاری پنیر موزارلا است می‌تواند نشان دهنده تازگی آن نیز باشد.

**واژه‌های کلیدی:** ژلاتین، پنیر موزارلا، صمغ ایرانی، فیلم حساس بهpH، عصاره هویج بنفش.

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