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INVESTIGATE MICROBIAL, CHEMICAL PROPERTIES AND SENSORY EVALUATION OF OSTRICH MEAT IN BILAYER FILMS CONTAINING POMEGRANATE PEEL POWDER DURING REFRIGERATED STORAGE, AS AN ACTIVE PACKAGING

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ABSTRACT

Pomegranate peel is a by-product which has antioxidant and antimicrobial properties and using it as an additive to improve the meat products shelf life in active packaging has increased in the last decade. The purpose of current study is to investigate the utilization of pomegranate peel powder (PPP) in bilayer film to increase the shelf life of ostrich meat (OM) at 4±1°C. In this research, OM was placed in bilayer films with different ratios of PPP (1,3,5%) then chemical and microbial experiments alongside sensory evaluation on OM were conducted on storage days (1,3,5,7,10). The results indicated that the values of chemical properties including pH, lipid oxidation and nitrogen, microbial, and sensory evaluation after 10 days in samples containing 5% PPP were lower than other samples. However, they had an undesirable color; Thus, the samples containing 3% PPP were preferred. This film was proposed as an active packaging for storing OM.

Keywords: Ostrich meat, Pomegranate peel powder, Film, Active packaging

INTRODUCTION

OM is one of the most delicious foods in Western societies due to its desirable nutritional properties (low cholesterol, muscle fat content, a high percentage of muscle fatty acids, and a high percentage of unsaturated fatty acids) therefore it has been marketed as a healthy alternative to the red meat. OM shelf life in retail is limited due to initial microbial loads and high pH **(Capita *et al.*, 2018)**. Novel packaging technologies can provide enhancement of the packaged product's shelf life by inhibiting or decreasing microbial growth **(Heydari *et al.*, 2020)**.
Antimicrobial compounds can be fixed, coated and place on the surface or applied to the packaging materials. Furthermore, antimicrobial compounds can be added in the form of laminated films for controlled release on the food surface **(Ahmed *et al.*, 2017)**. Numerous researches have demonstrated that the processing of vegetables and fruits a large amount of residue, like fruit peel. Also, it has been discovered that these by-products are highly rich in nutrients, primary biopolymers, and bioactive compounds **(Aali *et al.*, 2017; ghara gheshlagh Alireza *et al.*, 2018; He *et al.*, 2019; Mahmoudzadeh *et al.*, 2017)**.
Chemical and microbiological changes are the main reason of deterioration in the quality of meat. For this purpose, natural or artificial additives are used to increase the shelf life by preserving the quality of meat products. However, there are concerns and limitations about the use of artificial additives, as recent scientific studies have shown potential poisonous impacts and high costs, as well as growing consumer concerns about food additives. Fruits and vegetables are rich sources of valuable bioactive substances, particularly antioxidants, which can be considered a source of natural preservatives for meat products **(Abdel Fattah *et al.*, 2016; Kazeminia *et al.*, 2017)**.
Punica granatum (Punicaceae) known as the pomegranate, is a small tree which is native to the Mediterranean region **(Berizi *et al.*, 2016)**. Iran is one of the most significant producers and exporters of pomegranate in the entire world **(Basiri *et al.*, 2015)**. Pomegranate peel (PP) is an inedible section which is obtained during the processing of pomegranate juice and can be considered a by-product **(Berizi *et al.*, 2016; Berizi *et al.*, 2018)**.
PP has various antifungal, antimicrobial, and antibacterial activities, even though it is considered agricultural waste **(Bertolo *et al.*, 2020)**. The most abundant polyphenols present in PP are alginates, which can be hydrolyzed to alginic acid, and inhibits microbial growth if exposed to strong acids or microorganisms **(Ahmed *et al.*, 2017)**.
Fish gelatin can be obtained from cephalopods and fish skins, which is a by-product of the aquaculture and fisheries industry **(Kchaou *et al.*, 2018)**. And also, can be applied in the food industry due to its good film-making attributes and resistance to drying, light, and oxygen. It has become more popular as a coating material **(Feng *et al.*, 2017)**. Nevertheless, fish gelatin film has relatively weak water barrier properties and resistance, which is one of the main drawbacks of application in the use of packaging materials **(Hosseini *et al.*, 2016; Wu *et al.*, 2014)**.
As a consequence of the low melting temperature of fish gelatin, functional attributes can be simply modified by combining other active components for instance extracts and essential oils of the plants. Thus, Fish gelatin is the most proper carrier for fruit skin lesions, which allows direct laminating on polyethylene layers without removing the active ingredients in the preparation and coating stage **(Hanani *et al.*, 2018)**.
Polyethylene (PE) film is one of the common foods packaging because of its outstanding mechanical properties, low price, low moisture content, and excellent properties to prevent moisture penetration; however, it lacks the ability in view of the fact that it cannot be combined directly with active ingredients.
Due to the procedure of processing PE, which is frequently done at high temperatures, can cause the loss of active ingredients **(Hanani *et al.*, 2018; Khalid *et al.*, 2018)**. Thus, these active ingredients are frequently combined with another carrier and then layered on the PE surface to create a laminated film system **(Hanani *et al.*, 2018)**. The aim of the current study is to investigate the microbial, chemical attributes and sensory evaluation of OM in a bilayer film containing Pomegranate peel powder (PPP) during storage at refrigerator temperature, as an active packaging.

MATERIALS AND METHODS

Preparation of pomegranate peel powder

The pomegranate fruit of Saveh sweet-and-sour variety was purchased from Saveh city and then transferred to the school of health food hygiene and safety laboratory of Qazvin University of Medical Sciences. The pomegranate was washed with distilled water and then dried. The peel of the pomegranate fruit was peeled thereupon dried in an oven (made in Iran) at 30 °C for 24 hours. The dried peels were powdered by a shredder (made in Iran).

Preparation of bilayer films

To prepare five films for the experiments, the main base of the film was made at first; for this purpose, fish gelatin (Sigma Aldrich, USA) added 6% (w/v) into distilled water, and the solutions were stirred for 30 min at 900 rpm at a temperature of 50 °C. Then, 30% (w/w) glycerol (Merck, German) was added afterward the solutions were stirred for another 30 min. PPP with concentrations of 1%, 3%, and 5% (w/w based on gelatin film) was added to the film solutions. The film solutions were stirred continuously at 500 rpm for an additional 30 minutes at the room temperature. Another solution film was used as a control without adding PPP. The dried bilayer film was picked up from the plate and kept under a laboratory Cabinet at a temperature of 23±2 °C and Relative Humidity (RH) of 50% ± 5% **(Hanani *et al.*, 2018)**.

Preparation of ostrich meat samples

The fresh OM was bought from a specific store in Qazvin city and then under hygienic conditions and beside the ice transferred to the food safety and health laboratory. After washing it with distilled water in sterile conditions and below a laboratory hood (made in Iran) OM was divided into 5 g pieces. About 35 g of OM samples were put in each film to perform microbial, chemical experiments, and sensory evaluation. Packaging films include control film (without gelatin layer and PPP), film Polyethylene/ fish gelatin (PE/G) (without PPP), bilayer film (PE/G) containing 1, 3, and 5% PPP respectively and then OM were put in packaging and stored at of 4±1 °C for 10 days. Relevant experiments were conducted in three repeats on days 1, 3, 5, 7, and 10 of OM samples.

Bacteriological analyses

The packaging samples were opened under the laboratory hood and in sterile conditions. Then 10 g of the sample were taken with sterile forceps and placed in a sterile bag with 90 ml of sodium chloride (NaCl) 0.85% (Merck, Germany) which was homogenized. The Pour method was used for microbial culture (QUELAB, Canada). The microbial evaluations performed included total viable counts (TVC) (incubate at 37 °C for 2 days) and psychrotrophic bacterial counts (PTC) (incubate at 10 °C for 7 days) **(Capita *et al.*, 2018; Nowzari *et al.*, 2013)**.

pH analyses

On test days, five g of meat sample with 45 cc of deionized distilled water homogenized with a homogenizer for 1 min at a speed of 1000 rpm and then pH was measured using a Digital pH meter (Made in Iran, Model B2000) **(Mehdizadeh, 2018)**.

Determination of TBARS Analyses

To determine the thiobarbituric acid reactive substance (TBARS), the first 5 grams of the meat with 100 ml of 10% thiobarbituric acid solution (Merck Germany) were completely homogenized in a 250 ml beaker. Then the homogenized solution was passed from Whatman No. 42 filter paper (Merck, Germany). Three ml of the filtered solution with 3 ml of 0.02 trichloroacetic acid solution (Merck, Germany) were mixed together in a test tube and the mixture was placed in a water bath at 100 °C for 45 min. After cooling the samples, the absorbance of the pink solution was measured at 532 nm using a spectrophotometer (HACH Company, Germany). To prepare a control sample, 3 ml of 10% trichloroacetic acid solution was mixed with 3 ml of 0.2 M thiobarbituric acid solution. The results were expressed as mg of malonaldehyde/kg of a sample using the following formula **(Fazlara *et al.*, 2017; Ojagh *et al.*, 2010)**:

TBARS (mg MDA/Kg of tissue) $=\frac{50\times(A-B)}{200}$

A: The absorbance amount of the sample,
B: The absorbance amount of the standard thiobarbituric acid solution

Determination of TVN Analyses

Total volatile nitrogen (TVN) content is extensively used as an index for protein disintegration by microorganisms as well as protein breakdown by tissue proteolytic enzymes during storage **(El-Nashi *et al.*, 2015)**. To measure TVN at first Magnesium oxide was added to the homogenized OM sample then the distillation process was performed in a flask containing boric acid and methyl red. The boric acid solution was titrated with a sulfuric acid solution (H_2SO_4). The amount of TVN was obtained from the following formula **(Saki *et al.*, 2018)**:

TVN value ¼ 14V
V = ml of sulphuric acid (H_2SO_4) solution for titration

Sensory evaluation

To evaluate the sensory characteristics of raw OM, four important characteristics including texture, color, odor, and overall acceptability were examined. Features such as the absence of glaze on the surface of the muscle and the rapid return of the muscle to its original state and having a bright cherry red color and natural ostrich smell are among the superior sensory features **(Fazlara *et al.*, 2017)**. In this research, a 9-point hedonic evaluation was used (1=dislike extremely to 9=like extreme) **(Capita *et al.*, 2018)**. The panelists were staff and students of the Faculty of Health of Qazvin University of Medical Sciences and they were asked to rate the samples on a scale of 1 to 9 for these four characteristics.

Statistical analysis

Analysis of variance was used to compare mean differences of the samples by SPSS Ver. 22. Duncan's multiple range tests and critical difference were determined at the 5% significance level for comparing the means to find the difference between treatments and storage period ($P<0.05$).

RESULTS AND DISCUSSION

Effect of PPP on pH during refrigerated storage

According to Figure 1, the effect of pH on different days and samples indicates an increasing trend in all samples. The obtained data show that the lowest and highest pH amounts were related to samples with a PPP of 5% and also samples control, 6.54±0.17, and 7.77±0.07 during 10 days of storage, respectively ($p<0.05$). In samples containing PPP a significant decrease in trend pH ($p<0.05$) during storage were observed. In the continuation, they have had an increasing trend however, their amount in samples containing PPP is lower than the control sample. There is no significant ($p<0.05$) difference in pH between the control sample and the film PE/G sample. The pH of OM was 6.06±0.14 on time zero, which is similar to the results of the study which measured the pH of OM at 6.14±0.04 on time zero **(Fazlara *et al.*, 2017)**. The results indicate that fish gelatin has no significant impact on the pH of the samples. It can be concluded that the pH decreases in samples containing PPP is due to the low acidity of PPP. Another study about the effect of using pomegranate peel extract (PPE) and artichoke leaf extracts on improving the quality of marinated sardine fillets has shown that the pH in the samples containing PPE has the lowest value compared to the other samples **(Essid *et al.*, 2020)**. Moreover, the results of another research revealed that there is a significant decrease ($p\leq0.05$) in the pH of both samples containing PPP (2.5 and 5%) compared to the control sample **(Saleh *et al.*, 2021)**, which is in agreement with the present study that samples containing PPP have pH lower. Another researcher investigated that the pH in PP nanoparticles of 1.5% was less than in other samples during storage. The results show that the increase in pH is due to the breakdown of nitrogen compounds by endogenous or microbial enzymes **(Morsy *et al.*, 2018)**. The obtained data in this paper indicate an affirmative effect in reducing the pH of OM in packages containing PPP; In other words, the decreases in pH of samples in the products can have a positive effect on delaying the chemical and microbial spoilage of OM.

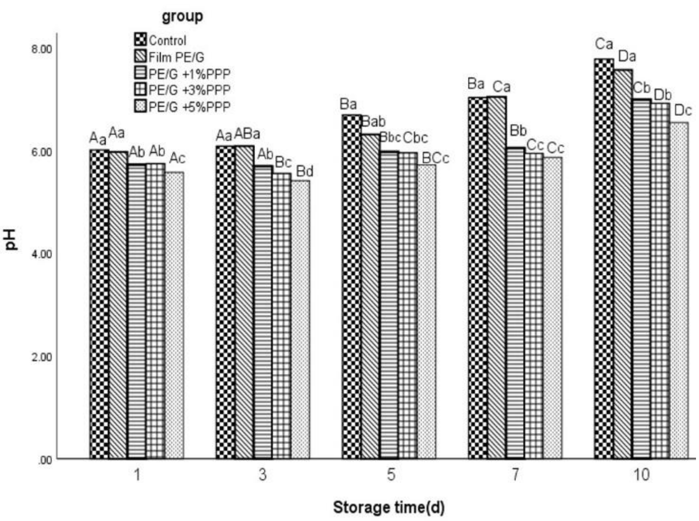

Figure 1 Changes in pH values of the samples prepared with different ratios of PPP during refrigerated storage at 4±1°C for 10 days.
Different small letters (a, b, c, d) are shown significant effect in different packaging groups ($p\leq0.05$)
Different capital letters (A, B, C, D) are shown significant effect in different storage period ($p\leq0.05$)

Effect of PPP on TBARS during refrigerated storage

Changes in TBARS values are shown in Figure 2. The values of TBARS have increased in all of the samples after 10 days of refrigerated storage, the TBARS level in the experimental samples containing PPP was significantly lower than in control samples and also film PE/G ($P<0.05$) samples. The lowest amount of malonaldehyde was observed in the sample containing 5% PPP 2.893±0.19. The highest for the film PE/G sample was 7.68±0.18 and the control sample was 7.28±0.08 during the storage period. TBARS are aldehydes which are produced following the breakdown of lipid peroxidation products such as malondialdehyde **(Bouarab-Chibane *et al.*, 2017)**. Three mg of malondialdehyde per kilogram have been reported as oxidative spoilage in poultry meat **(Fazlara *et al.*, 2017)**. In the present study, this report has been used for the OM spoilage. There is no significant ($P\leq0.05$) difference between control and film PE/G samples.
These samples were permissible in the range until the third day of storage. The samples containing 1%, 3%, and 5% PPP were within the acceptable range until the 7th, 7th, and 10th day, respectively. A study showed the samples containing 2 to 0.50% (w/w) PPE in shrimp of minced meat significantly ($P\leq0.05$) inhibited TBARS in 28 days of storage **(Ismail *et al.*, 2019)**. The result of another perusal, which the effect of PPP on beef sausage stored at -18 ± 2°C, revealed that the lowest amount of TBARS is recorded for beef sausages contained 5% of PPP, while the highest amount was in the control sample **(Saleh *et al.*, 2021)**. In a similar study have examined the impact of PPP on the characteristics of beef burgers in a refrigerator for 12 days, and resulted that the TBARS in samples containing different concentrations of PPP (1%, 2%, 3%) were less than the range of permissible level after 12 days of storage, however in the control sample was above permissible level range after 12 days **(Abdel Fattah *et al.*, 2016)**. These results are mentioned consistently with the results of current study which demonstrates the effect of adding PPP in the inhibition of TBARS.

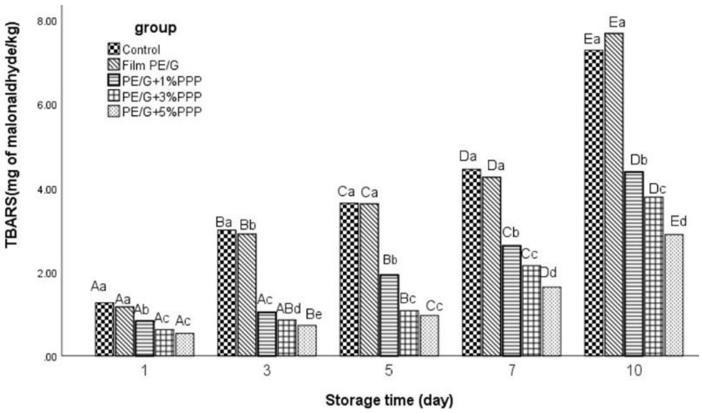

Figure 2 Changes TBARS OM samples during refrigerated storage.
Different small letters (a, b, c, d) are shown significant effect in different packaging groups ($p\leq0.05$)
Different capital letters (A, B, C, D) are shown significant effect in different storage period ($p\leq0.05$)

Effect of PPP on TVN during refrigerated storage

The results showed that the values of TVN in all samples are increasing. As a matter of fact, this procedure in samples containing different amounts of PPP proceeds with a slower slope. The findings are shown in Figure 3. Correspondent to the research results, the highest and lowest TVN are related to the control sample with 45.09±3.43 and the sample contains 5% PPP 25.44±1.32, respectively. There is no remarkable difference ($P\leq0.05$) in TVN between control and film PE/G samples. In this research, the values of TVN in samples with PPP are less than in control and film PE/G samples. The TVN results are consistent with TVC and PTC consequences in that their values were lower in samples containing PPP. This can be due to the prevention of microbial growth and the consequent non-decomposition of proteins by bacteria. According to the results of the current study, the presence of fish gelatin did not impact TVN in samples, and the PPP has caused a reduction in values of TVN. In a research the effects of PP nanoparticles on meatballs during refrigerated storage were investigated that meatballs with 1.5% LP-NPs (lyophilized PP nanoparticles) have the lowest TVN value compared with 1% LPP-NPs and BHT(butylated hydroxytoluene) during storage **(Morsy *et al.*, 2018)**. Another research has been done about the effects of PPP on beef burgers during a storage period in a refrigerator. The results indicated that in the control sample, this rate at the time of zero was 8.89 mg/100g, and then has increased to 54.25 mg/100g at the end of the 12day. The values of the beef burger sample containing 4% PPP had the lowest amount which was 8.27 mg/100g at the beginning of the storage period and 19.51 mg/100 at the end of the storage period after 12 days**(Abdel Fattah *et al.*, 2016)**. This result was consistent with the current study. In another study, the values of TVN in beef sausage samples containing 2.50% and 5% PPP, were 16.0±3.45 and 14.33±4.21, respectively, and in the control sample was 20.04±4.10, after 8 weeks which showed a slow increase in treatments containing PPP **(Saleh *et al.*, 2021)**. The obtained data have resembled the present study.

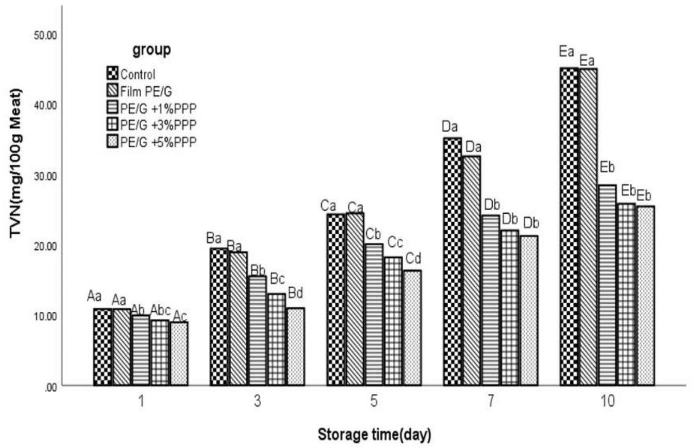

Figure 3 Changes TVN OM samples during refrigerated storage.
Different small letters (a, b, c, d) are shown effect significant in different packaging groups ($p\leq0.05$)
Different capital letters (A, B, C, D) are shown effect significant in different storage period ($p\leq0.05$)

Effect of PPP on microbiological analyses total TVC and PTC during refrigerated storage

According to the (ICMSF) Statement in 1986, the maximum limit for consumption of total viable bacterial count (TVBC) is 7 log CFU/g, and reaching the logarithmic mean of bacteria to this extent is expressed as the beginning of meat spoilage **(Fazlara *et al.*, 2017; Feng *et al.*, 2017)**.
In this study microbial indicators are TVC and PTC, the trend of changes in different samples during refrigerated storage at 4±1°C for 10 days are shown in Figures 4 and 5. The trend of changes in TVC and PTC increased in all samples regarding TVC bacteria, the lowest and highest after 10 days of storage were in the sample containing 5% PPP, 8.10 ±0.10, and in the control sample 8.65±0.22, respectively. In PTC, the lowest and highest microbial counts belonged to the containing 5% PPP sample and the control sample 8.10±0.10 and 8.65±0.22, respectively ($p<0.05$). Pursuant to the results obtained on the count of TVC bacteria in the samples, the allowed value in the control sample was on the third day (4.34±0.49), film PE/G sample until the fifth day (6.97±0.36), Samples containing 1% and 3% PPP were respectively 6.54±0.41 and 5.94±0.09 until the seventh day of storage and in the sample containing 5% PPP, the bacterial count on the tenth day reached the allowable range (7.00±0.36). Psychrotrophic Permissible microbial counts in control and film PE/G samples were respectively 5.43±0.05 and 5.59 ± 0.34 until the fifth day of storage. In the sample containing 1% PPP on the seventh day, it reached the permissible limit of 7.00±0.21. Also, in samples containing 3% and 5%, PPP were 6.94±0.06 and 6.87±0.05, respectively. The results were less than the allowed value on the seventh day. In the film PE/G samples, the effect of fish gelatin is not very remarkable for inhibiting microbial growth. A research about the effects of gelatin- Avishan Shirazi on ostrich fillets showed that the coating containing Avishan Shirazi extract with gelatin had the greatest effect in reducing microbial load compared to Avishan Shirazi or gelatin alone, i.e. gelatin had no effect by itself **(Fazlara *et al.*, 2017)**. Another study reported that the bacteriological test of shrimp bacterial populations wrapped in gelatin films was significantly higher than in chitosan films, indicating that the pure fish gelatin films had no impact on prolonging the shelf life of wrapped shrimps. The low protective role of gelatin is only due to the formation of a physical barrier against the penetration of microbes **(Mohebi and Shahbazi 2017)**. Moreover, another study reported that the amount of TVC in marinated sardine fillets samples with pomegranate peel extract (PPE) was lower than in artichoke extract, which indicates the strong antimicrobial properties of PPE compared to artichoke leaf extracts **(Essid *et al.*, 2020)**. Another study showed that the TVC of bacteria at samples containing beef sausages treated with ratios of 2.50% and 5% of PPP had a notable decrease ($p\leq0.05$) in the second and third weeks **(Saleh *et al.*, 2021)**. This is consistent with the results obtained in this study which shows that an increase in the concentration of PPP leads to a reduction in the count of bacteria. In a study about the effects of methanolic pomegranate peel extract (MPPE) on the characteristics of Gutted Rainbow Trout during frozen storage were examined. Based on the results, the sample with 4% MPPE has the lowest number of PTC at the end of storage **(Berizi *et al.*, 2016)**. In another study impact coated rainbow trout by chitosan combined with different ratio of PPE during frozen storage was showed. In chitosan+PPE2%, and chitosan+PPE4% samples, the amount of other PTC were less than in the control sample, and even less than chitosan sample, which indicates the strength of PPE in microbial inhibition **(Berizi *et al.*, 2018)**.

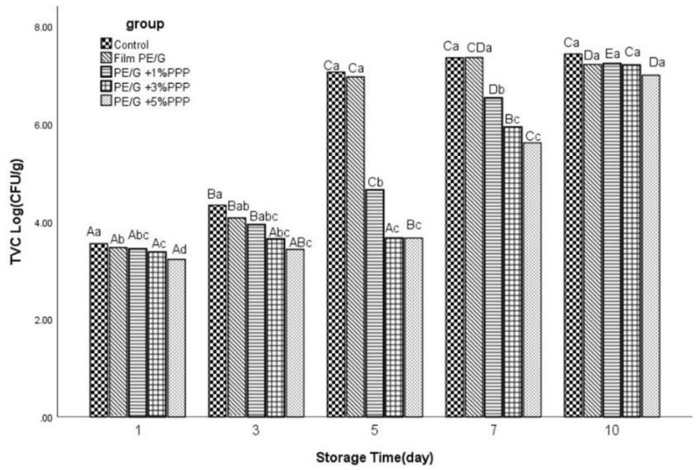

Figure 4 Changes in TVC OM samples during refrigerated storage.
Different small letters (a, b, c, d) are shown significant effect in different packaging groups (p≤0.05)
Different capital letters (A, B, C, D) are shown significant effect in different storage period (p≤0.05)

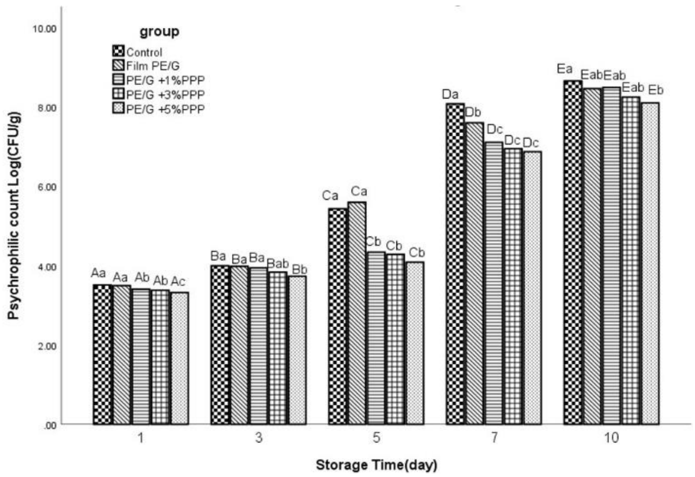

Figure 5 Changes in PTC OM samples during refrigerated storage.
Different small letters (a, b, c, d) are shown significant effect in different packaging groups (p≤0.05)
Different capital letters (A, B, C, D) are shown significant effect in different storage period (p≤0.05)

Sensory evaluation

Sensory characteristics including color, texture, odor, and overall acceptability of OM were examined. Samples containing various concentrations of PPP were tested and the results are shown in Table 1. According to the color evaluation, the highest score was for the sample with 1% PPP and the lowest scores were for the sample with 5% PPP and the control sample after 10 days of storage in the refrigerator temperature (p≤0.05). In the odor evaluation to, the highest scores were related to the samples containing PPP and the lowest score was related to the control sample. In the sensory evaluation of the texture of the samples, the lowest scores belonged to the control sample and the highest scores were belonged to the sample with 5% PPP. In the sensory evaluation about the overall acceptability, the samples with 5% PPP had the highest scores, and the lowest score was related to the control sample after 10 days of storage in the refrigerator. With increasing PPP concentration and storage, the color of the product became darker and this was not pleasant for the consumer.

It is noteworthy, that the use of 1 and 3% concentration of PPP have shown the less opacity color in the present research. Another study abreast with the present study shows that MPEE is a colored and aromatic component, the increase in unpleasant color and odor of samples treated with higher ratios of MPEE was not astonishing **(Berizi *et al.*, 2016)**. A study on the evaluation sensory of samples containing pomegranate and artichoke peel extracts showed a darker appearance in samples containing pomegranate and artichoke peel extracts than in control, due to penetration of the extracts into the fish tissue causing the fish to be darker. However, in the end, the highest scores in overall acceptability were belonged to the sample containing PPE compared to other samples **(Essid *et al.*, 2020)**. The same result was observed in another study that which sensory evaluation showed that LPP-NP had no negative effect on the sensory attributes of cooked and raw meatballs **(Morsy *et al.*, 2018)**.In addition, in a study, the effect of the sensory evaluation was examined chitosan with PPE in rainbow trout during frozen storage. Based on the findings, the best evaluations are related to samples with PPE i.e. chitosan+PPE2 and chitosan+PPE4 **(Berizi *et al.*, 2018)**.

Table 1 Sensory evaluation on OM samples prepared with different ratios of PPP during refrigerated storage at 4±1°C for 10 days.

Parameter	Day	Control	Film PE/G	Sample 1	Sample 2	Sample 3
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Color	1	$8±0.65^{Aa}$	$7.30±0.96^{Ab}$	$7±0.75^{Ab}$	$6.30±0.88^{Ac}$	$5.93±0.88^{Ac}$
	3	$6.87±0.83^{Ba}$	$6.53±0.83^{Ba}$	$6.50±0.83^{Aa}$	$5.80±0.86^{Ab}$	$4.93±1.10^{Bc}$
	5	$4.53±0.92^{Ca}$	$4.86±0.99^{Ca}$	$4.53±0.83^{Ba}$	$3.50±0.83^{Bb}$	$3.40±1.40^{Cb}$
	7	$4.33±1.18^{Ca}$	$5.10±0.88^{Cb}$	$4.80±0.01^{Bab}$	$3.30±0.79^{Bc}$	$2.30±0.70^{Dd}$
	10	$2.80±0.77^{Da}$	$3±1.10^{Da}$	$4.40±0.98^{Bb}$	$2.6±0.63^{Ca}$	$1.8±0.77^{Dc}$
Odor	1	$6.87±0.83^{Aab}$	$6.67±0.62^{Aa}$	$6.93±0.70^{Aab}$	$7.93±0.59^{Ac}$	$7.40±0.83^{Ab}$
	3	$4.80±1.10^{Ba}$	$5.33±0.82^{Ba}$	$6.50±0.83^{Ab}$	$6.26±0.88^{Bb}$	$6.40±0.83^{Bb}$
	5	$3.30±0.80^{Ca}$	$3±0.76^{Ca}$	$5.10±0.88^{Bb}$	$5.53±0.99^{Ccb}$	$6±0.84^{Bc}$
	7	$1.90±0.64^{Da}$	$2.30±0.70^{Da}$	$3.33±0.82^{Cb}$	$4.26±0.88^{Dc}$	$5.10±0.88^{Cd}$
	10	$1.13±0.40^{Ea}$	$1.60±0.51^{Eb}$	$3.10±0.59^{Cc}$	$3.60±0.63^{Ed}$	$4.10±0.70^{De}$
Texture	1	$7.80±0.80^{Aa}$	$7.50±0.64^{Aa}$	$7.86±0.83^{Aa}$	$8±0.65^{Aa}$	$8.10±0.88^{Aa}$
	3	$6.20±0.80^{Bab}$	$5.53±0.92^{Ba}$	$6.50±0.92^{Bb}$	$6.60±.98^{Bb}$	$6.70±0.98^{Bb}$
	5	$5.33±0.82^{Ca}$	$5.10±1.03^{Ba}$	$5.20±1.21^{Ca}$	$6.06±0.98^{Bb}$	$6.90±0.99^{Bb}$
	7	$2.93±0.96^{Da}$	$2.50±0.83^{Ca}$	$4.73±1.10^{Cb}$	$5.33±0.82^{Ccb}$	$5.70±0.96^{Cc}$
	10	$1.46±0.64^{Ea}$	$1.50±0.52^{Da}$	$3±0.76^{Db}$	$4±0.75^{Dc}$	$4.80±0.86^{Dd}$
Acceptability	1	$7.9±0.74^{Aa}$	$7.40±0.74^{Aab}$	$7±0.84^{Ab}$	$7.60±1.10^{Aab}$	$7.40±0.63^{Aab}$
	3	$6.40±0.83^{Ba}$	$6.40±0.74^{Ba}$	$6.33±0.72^{Ba}$	$6.30±0.70^{Ba}$	$5.70±1.04^{Bb}$
	5	$3.50±0.92^{Ca}$	$3.20±0.94^{Ca}$	$4.33±1.05^{Cb}$	$5.10±0.88^{Cc}$	$5.90±0.83^{Bd}$
	7	$3.10±0.70^{Ca}$	$3.33±0.89^{Cab}$	$3.80±0.94^{Cbc}$	$4.33±0.97^{Dc}$	$5±0.92^{Cd}$
	10	$1.33±0.49^{Da}$	$1.30±0.46^{Da}$	$2.50±0.74^{Db}$	$3.13±0.64^{Ec}$	$3.70±0.90^{Dd}$

Different small letters (a, b, c, d) are shown effect significant in different packaging groups (effect of treatments) (p≤0.05)
Different capital letters (A, B, C, D) are shown effect significant in different storage period (p≤0.05)

CONCLUSION

According to the results of the present study, the presence of film containing fish gelatin without PPP has no notable effect on the inhibition of microbial growth, lipid oxidation as well as reduction of pH, TVN, and sensory attributes of OM.
On the report of the experimental results, film samples containing PPP retain the microbial, chemical, and sensory attributes of OM and increase the shelf life of the product at refrigerator temperature. The presence of PPP slightly reduces the pH of OM, which causes a reduction in the growth of bacteria and also a reduction in nitrogen production due to bacterial activity and thus reducing the amount of TVN. The antimicrobial and antioxidant properties of PPP reduce microbial growth, TBARS, and TVN. Also, by increasing the ratio of PPP in bilayer films, the sensory quality of OM has been improved, which has increased the shelf life of the product in the refrigerator for at least 7 days. Finally, the use of PPE as a natural additive is recommended to increase the shelf life of OM. For future studies, the use of other meats as a sample as well as the use of biodegradable film instead of polyethylene film is suggested.

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