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Oxidative Stability of Whey Protein Isolate Coating Incorporated Beeswax: A Shelf-Life Study in Sliced Pastirma

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Abstract | The main goal of the current study was to apply the whey protein isolate/beeswax (WPI/BW) as an edible coating to improve the quality and extend the shelf life of sliced pastirma stored under either aerobic or vacuum packaging. Pastirma was produced, sliced, and then allocated to four groups; the first group was aerobically packed and used as control, the second group was vacuum packed, while the third and fourth groups were coated with WPI/BW then packed in either polyethylene or vacuum bags. All groups were stored at 5 ˚C until the signs of unacceptability were noticed. The results showed that WPI/BW coated pastirma stored under vacuum packaging showed the most oxidative stability (low TBARS, peroxide values, met-myoglobin, and carbonyl contents, high scavenging activity) and the highest sensory quality among the different treatments. The composite treatments extended the wholesomeness of pastirma to 16 weeks at 5 ˚C in comparison with 12, 8, and 4 weeks for uncoated vacuum-packed, aerobically packed WPI/BW, and untreated control samples, respectively. Therefore, the application of WBI/BW edible coating before packaging of pastirma may be safely used at a commercial scale to extend the durability of pastirma.

Keywords | Pastirma, Whey protein, Beeswax, DPPH, carbonyl compound.

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INTRODUCTION

Fat oxidation is the main cause of non-microbial degradation in meat products, it abridges shelf life, degrades sensory quality, and increases consumer rejection. Moreover, lipid degradation end-products lead to protein oxidation and the development of carbonyl derivatives that harm the color of meat products (Xiong and Guo, 2021). The oxidation of meat products is closely correlated with its complicated nutritional composition and storage conditions. Heme-proteins, metals, enzymes, and common salt, all promote fat oxidation. The concentration of heme-proteins differs according to the species and location of the muscle, where muscles with high myoglobin contents are more liable for lipid oxidation (Bekhit et al., 2019), sodium chloride has a pro-oxidant effect (Amaral et al., 2018) and exposure to oxygen accelerates the development of rancidity (Chaijan and Panpipat, 2017).

Pastirma is a protein-rich (25%) traditional dry-cured meat product prepared from high-quality intact muscles of the hind-quarter. Pastirma production involved several

stages and finally, pastirma is stabilized by the presence of high salt content and reduction in the water activity, however, it is vulnerable to both microbial degradation as well as chemical deterioration due to its neutral pH (6), relatively high moisture content (60%), high hem pigments in the raw meat material, and high salt content in the finished product (8%) (Abdallah et al., 2017). The intact pastirma can be safely stored for one month at 25°C while slicing raises the risk of microbial contamination, which degrades the quality and reduces the shelf life as a consequence of fat oxidation as well as microbial proliferation. In response to recent and enhanced consumer satisfaction with chemical additives, additional novel preservation technologies must be appointed to extend shelf life, retain protection, and preserve the food.

Vacuum packaging can securely preserve dry-cured meat products and maintain a professional presentation during storage shelf life, however, the growing lactic acid bacteria "LAB" reduces their acceptability due to purge loss, color oxidation, off-flavor, and slimy appearance (Pothakos et al., 2015). Edible coatings can represent a golden preservation technique to overcome these problems. Protein, carbohydrate, and lipid edible coating are widely used in food preservation. The most attractive protein used is the whey protein isolate (WPI), which has good nutritional and functional properties, excellent gas barrier properties, and the ability to form a transparent flexible coat of good mechanical strength (Song et al., 2020), but it unfavorably affects the physical properties of the food as a result of moisture loss due to its hydrophilic nature (Kouracand et al., 2020). Therefore, the use of composite technology e.g., incorporation of lipids as beeswax may be an innovative solution to improve water vapor permeability, oxygen, and light barrier characteristics (Pérez-Vergara et al., 2020).

The food industry is interested in meat product oxidative stability, which is a dynamic multifactorial phenomenon that has significant implications for both wholesomeness and quality. Since oxygen is the fundamental factor in lipid degradation, reducing the oxygen tension in the packaging environment would be an effective method for limiting meat oxidation. As a result, this study aimed to display the oxidation of lipid, protein, and heme pigments and their correlation with the sensory quality of sliced vacuum-packed pastirma and to identify reliable methods as a helpful step in improving the stability of vacuum packaged pastirma.

MATERIALS AND METHODS

Raw materials

Frozen beef topside muscles were obtained from a local supplier after 3 months of their production. WPI (92.0% protein, 1.0% lactose, 1.8% fat, and 3.5% ash content) was supplied by Davisco International, Inc. (BiPro, Le Seur, MN, USA). Glycerol was obtained from Loba Chemie, Mumbai, India. Beeswax (98% purity) was supplied by Somerset Company (Renton, WA, USA). Calcium chloride was bought from Sigma-Aldrich (USA).

Preparation of whey protein isolate/beeswax (WPI/BW) composite coating

For the preparation of WPI/BW edible coating, 6% WPI was dissolved in 0.04% $CaCl_2$ solution and 2% glycerol. After mixing, the pH was adjusted to 8.0 with 1.0 N NaOH, then heated at 90°C for 30 min in a shaking water bath. During the last 5 min of heating, 0.4% beeswax was added, and homogenized for 2 min, then filtered through a layer of gauze and cooled.

Processing, coating, packaging, and storage of sliced pastirma

Three replicates of pastirma were processed at different times following the procedure of Abdallah et al. (2017), then sliced into 2 mm slices, weighted into 200±5.00 g portions, and finally divided into four groups. The 1st group was packed in a clean transparent polyethylene bag (regular marketing presentation) and used as control. The 2nd group was packed in Low-Density vacuum bags (Vollrath, USA) and sealed using SD520 Komet vacuum packing machine (KOMET, Germany) for 30 s at 0.8 bar. The vacuum bags were 40 microns thick, 3.99 cc/100 in^2/24 hr oxygen transfer rate (65% RH, 23°C), and 0.54 g/100 in^2/24 hr (90% RH, 38°C) water vapor transfer rate. However, the 3rd and 4th groups were dipped in WPI\BW edible coating for 1 min, drained for 2 min on a perforated rack, and dried in an electrical hot air oven for 5 min using forced airflow without heating, then dipped for 1 min and dried once more. After the application of the coating, the sliced pastirma of the 3rd and 4th groups were packed in polyethylene and vacuum bags, respectively. All samples were stored at 5°C and sampling was conducted after 24 hr. (0-time) and every 14 days till become organoleptically unacceptable.

Examination of sliced pastirma

Sensory analysis

The sensory discriminative test was conducted on both coated and uncoated sliced pastirma to investigate its quality during storage with or without vacuum packaging. A total of 23 males and 27 females usual pastirma consumers (90% of them eat pastirma one time monthly at least) shared in the survey. For each storage time, 5 sessions were held on five independent days, where each consumer tested one sample from each group served in a random order. The overall sensory acceptability in addition to cured color, cured flavor, and taste was thoroughly defined, and the consumers allocate a score for each sensory parameter

on a numerical scale ranging from 1 (extremely dislike) to 9 (extremely like).

Bacteriological examination

For microbiological analyses, tenfold decimal dilutions were prepared according to ISO/6887-1 (2017). The total aerobic bacterial count was performed using Plate Count Agar (Oxoid CM0463) incubated at 35 ºC for 48 hours (Ryser and Schuman, 2015). Moreover, LAB was enumerated using DeMan Rogosa Sharpe agar plates **(**Oxoid CM 1153**)** incubated at 30 ºC under microaerophilic conditions (5% CO_2) for 3 days (de Man et al., 1960).

Protein oxidation (Total carbonyl)

One gram of each pastirma sample was homogenized with 10 mL of pyrophosphate buffer, 2 mM ethylene glycol tetra acetic acid, 2 mM $MgCl_2$ and 100 mM KCL using a stomacher (Lab blender 400). For precipitation of sample, 2 ml of homogenate was mixed with 2 mL of 20% TCA, and centrifuged for 5 min at 12000 ×*g*. After that, one part of the precipitate was dissolved with 2 mL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) and the other part was used as a blank by its mixture with 2 M HCl. Both the sample and the blank were kept in dark place for 30 min with intermitted shacking every 3 min. Then, the sample was mixed again with 2 mL of 20% TCA, and centrifuged for 5 min at 12000 ×*g* for precipitation of the protein. The protein precipitate then was washed three times with 4 mL of 10 mM HCl followed by centrifugation to remove DNPH. Finally, the precipitate was solubilized in 2 mL of 6 mM guanidine hydrochloride and kept at 5 °C for 12 hours. The protein concentration and total carbonyl content was obtained by measuring the absorbance at 280 and 370 nm, respectively after the subtraction of the absorbance values of the blank from the sample. The absorption coefficient of 22000/M/cm was used to calculate the carbonyl content, which expressed as nmol/mg protein (Lund et al., 2008).

pH and Total Volatile Basic Nitrogen "TVBN"

The pH values were measured using a Lovibond Senso Direct digital pH-meter (England) with a probe-type electrode (Senso Direct Type 330) in an aliquot prepared by blending 10 g pastirma in 100 ml distilled water for 15 s. Three reading for each replicate were obtained to calculate the mean pH value. The reference method of perchloric acid extraction adopted by the European Union (1995) was followed for the determination of TVBN content (mg/100g).

Lipid stability parameters (Tbars, Peroxide values, and free radical scavenging activity)

TBARS value (mg malonaldehyde/ kg) was measured according to the method established by Zhang et al. (2016), and the peroxide value "PV" (meq/kg fat) was measured following the procedures described by Shantha and Decker (1994). Though, the procedures reported by Brand-Williams et al. (1995) was followed for assessment of DPPH scavenging activity where, the sample homogenate for each pastirma treatment was prepared by homogenizing 13.3 grams of sample with 100 ml distilled water. From each sample homogenate, 2.5 ml were mixed with 20 ml ethanol, left for 20 minutes then completed up to 25 ml with ethanol and filtered. One and half ml freshly prepared DPPH solution (0.06 mM) was mixed with 1.5 ml diluted sample, allowed to react at room temperature for 30 minutes in dark place. Two and half ml distilled water were mixed with 22.5 ml ethanol and used as a blank. One and half ml freshly prepared DPPH solution was added to 1.5 mL blank and used as a control. The absorbance values of control and samples were measured at 517 nm against the blank, and the antioxidant activity percentage was determined using the formula of Mensor et al. (2001) as the following:

Color oxidation and Instrumental color indexes

Color oxidation was calculated as a percentage of the met-myoglobin to the total myoglobin content using the method of An et al. (2010). The color indexes on the surface of sliced pastirma samples were observed during storage by recording the CIE L^* (lightness), a^* (redness), and b^* (yellowness) values at angle of 10 degrees with illuminant D65 light source using Konica Minolta Chroma meter (CR 410, Japan) calibrated with a white plate and light trap. Readings were obtained 15 min after the opening of the package. Fifteen readings were obtained for each index and the mean was calculated.

Statistical analysis

Each experiment was done three times on different occasions and three samples from each replicate were analyzed at each sampling time. Data of sensory panel scores as well as lipid, protein, and color oxidation in addition to bacterial loads (Log_{10} CFU/g) of each pastirma trial, were analyzed using SPSS statistics 23.0 for windows. Comparison of the results between the different pastirma trials was carried out by using one-way analysis of variance (ANOVA) throughout the chilling storage for 16 weeks where, the significant differences were considered at $P<0.05$.

RESULT AND DISCUSSION

Sensory quality

Sensory evaluation not only revealed that coated pastirma stored by vacuum packaging received the highest ratings but also had extended sensory stability for 4 months in comparison with 3, 2, and 1 month for uncoated vac-

uum-packed, WPI coated aerobically packed, and finally untreated aerobically packed control samples, both immediately after processing and during storage at 5°C (Fig. 1A,B). The key explanation for the improved sensory quality was the powerful antioxidant activity and exceptional gas barrier properties of WPI coating (Song et al., 2020), which prevents color oxidation and the formation of oxidation-mediated off-flavors during storage. The higher color score of WPI/BW coated pastirma (Fig. 1A) may be also due to the gloss appearance provided by the beeswax (Trevisani et al., 2017). However, Jiménez et al. (2010) observed that the incorporation of lipids into the edible coating adversely affects the color of the meat surface which appeared unacceptable and cloudy. The lipophilic nature of flavor substances (Chiumarelli and Hubinger, 2012), and the barrier properties of WPI (Song et al., 2020) may explain the flavor stability of WPI/BW coated pastirma during storage (Fig. 1A). Moreover, the high moisture retention of beeswax coatings could explain the higher taste panel scores of coated pastirma either stored alone or in combination with vacuum packaging (Fig 1B) (García et al., 2000). Improving the cured color, cured flavor and taste by the combination between the application of WPI/BW coating and vacuum packaging during the processing of sliced pastirma made this trial more acceptable for the consumer, which indicated by the elevation of its over acceptability scores when compared with other pastirma treatments (Fig. 1B).

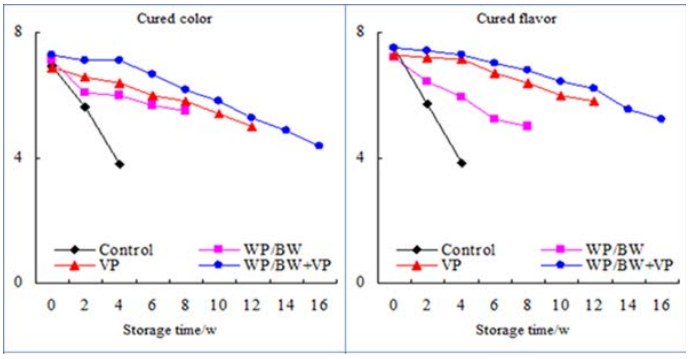

Figure (1A): Cured color and cured flavor of sliced pastirma stored at 5°C for 16 weeks

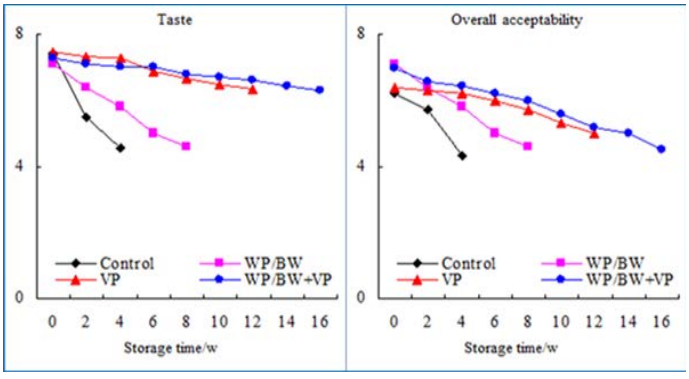

Figure (1B): Taste and overall acceptability of sliced pastirma stored at 5°C for 16 weeks

Bacterial quality

The aerobic bacterial count was relatively high (< 4.00 $\log_{10}$ CFU/g) on the next day after the application of edible-coat probably due to handling during slicing and coating. The bacterial load then increased in aerobically packed pastirma to reach an unacceptable count in the 1st month of storage at 5 °C in the control group compared with the 2nd month for WPI-coated pastirma. However, the count decreased by about 1 log in vacuum-packed treatments till the 2nd month then started to increase but didn't reach such high levels till the end of storage shelf life with marked privilege for WPI coated samples (Fig. 2). The lower count in WPI coated pastirma could be due to the broad-spectrum bactericidal effect of lactoperoxidase (Al-Baarri et al., 2011). Despite that storage of sliced pastirma under vacuum packaging is expected to maintain its sensory quality for 3 months of refrigeration there is a concern about the growth of LAB. The LAB populations immediately after processing is generally below < 3.00 $\log_{10}$ CFU/g and increased to about 7.00 $\log_{10}$ CFU/g in the control sample at the 1st month of storage. Nearly the same count was reached at about the 3rd month in vacuum-packed samples. However, the application of WPI coating resulted in a much slower increase in LAB count, and the combination of vacuum-packaging and WPI coat resulted in a linear decrease in the bacterial count till diminishing to below the detectable limit (< 2.00 $\log_{10}$ CFU/g) after 3.5 months of refrigeration storage (Fig. 2). The longer shelf span of vacuum-packed coated pastirma is due to the synergistic antibacterial effect and the gas and moisture barrier properties as well as the potential to limit the metabolic reactions (Shokri and Ehsani, 2019).

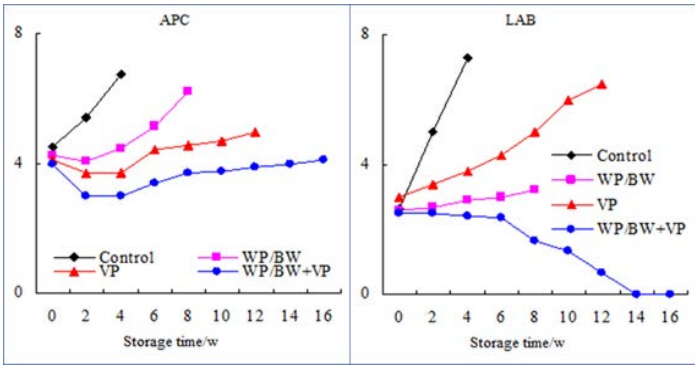

Figure 2: Bacteriological analysis ($\log_{10}$ CFU/g) of sliced pastirma stored at 5°C for 16 weeks

In general, bacterial growth was correlated with similar changes in oxidation criteria and sensory quality. In aerobically packed pastirma, the exposure to oxygen accelerated the progress of aerobic bacteria, which resulted in a change in both color and flavor (Fig. 1, 2), and increased the liability of lipid, protein (Fig. 3), and color oxidation (Fig. 4). Moreover, the growth of LAB in vacuum-packed sliced pastirma was correlated with the development of off-odor

and color deterioration (Pothakos et al., 2015).

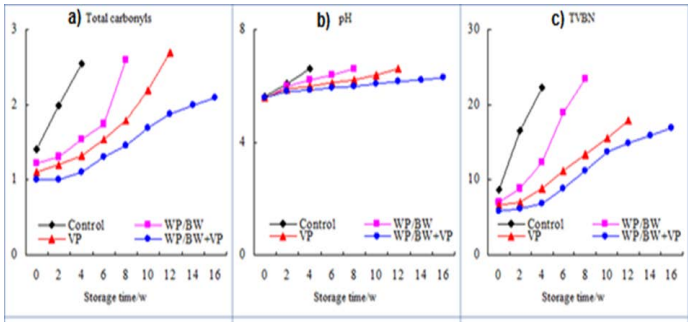

Figure 3: a) Total carbonyl (µmol/g protein), b) pH and c) TVBN (mg/100g) values of sliced pastirma stored at 5°C for 16 weeks

Protein oxidation

The total carbonyl compounds were low in all groups shortly after processing and increased significantly during chilling storage regardless of the packaging technique (Fig. 3a). Moreover, there were clear variations between the four investigated groups, with vacuum-packed samples having lower carbonyl contents than aerobically packed ones, and the combination of vacuum packaging and WPI coating exerted more inhibitory effects, resulting in a pronounced delay in carbonyls formation. It was also clear that the formation of carbonyls in all groups followed the same pattern of bacterial growth and the changes in both sensory and physico-chemical criteria of sliced pastirma, which substantiated the finding of Wongwichian et al. (2015) who found that the formation of carbonyls is related with the development of off-flavor, rancidity, met-myoglobin formation, and color deterioration.

pH and TVBN values

The pH values of aerobically packed uncoated pastirma were higher than the other treatments immediately after production and gradually increased during storage at 5°C to reach an unacceptable value (6.6) after only 3 weeks after the production, however, the same value was reached after 8, 12 and 16 weeks post-processing in WPI coated, vacuum-packed, and WPI/vacuum-packed samples, respectively (Fig. 3b) following the same pattern of aerobic bacteria (Fig. 2). The continuous increase in pH over time was more heightened in uncoated samples rather than in the coated ones and aerobically than vacuum packaged group. The rise in pH may be due to the buildup of bacterial metabolites in addition to protein, amino acid degradation, and the formation of ammonia (Ercolini et al., 2011).

The unacceptable flavor developed once the TVBN of pastirma exceeded 20 mg/100g, which occurred after the 3rd week of chilling storage in control treatment compared with the 6th week in aerobically packed coated pastirma, however, the sliced pastirma treated with vacuum-packaging and the combination with WPI coat didn't reach these values even at the 12th and 16th week of refrigerated storage, respectively (Fig. 3c). However, Zhu and Zhang (2004) found that off-flavor of dry-cured meat developed at 25 mg/100g but an unpleasant odor is produced if the TVBN value exceeds 100 mg/100g.

Lipid stability parameters

The lipid oxidation criteria of sliced pastirma indicated that better fat stability can be gained via synergistic strategies. Storage of WPI-coated pastirma using vacuum packaging resulted in the lowest TBARS and PV among the different treatments after processing and during chilled storage (Fig. 4 a, b). The mean TBARS and PV increased linearly and remained within the acceptable limit (0.9 mg/kg and 20 meq/kg, respectively) until the 16th week of storage compared with the 10th, 8th, and 4th week for vacuum-packed, coated, and uncoated control groups, respectively. The improvement of antioxidant activities due to coating of sliced pastirma before vacuum packaging has been verified by the measurement of DPPH. The free radicals scavenging activities "DPPH" indicated that vacuum-packed WPI-coated pastirma exhibited the highest antioxidant activity, while the aerobically packed control samples showed the lowest radical scavenging activities (Fig. 4 c). In general vacuum packaging exerted much more scavenging activity in comparison with aerobic packaging. The noticeable high antioxidant characteristics of WPI may be due to its good oxygen barrier properties and free radical quenching activity (Zhang et al., 2020).

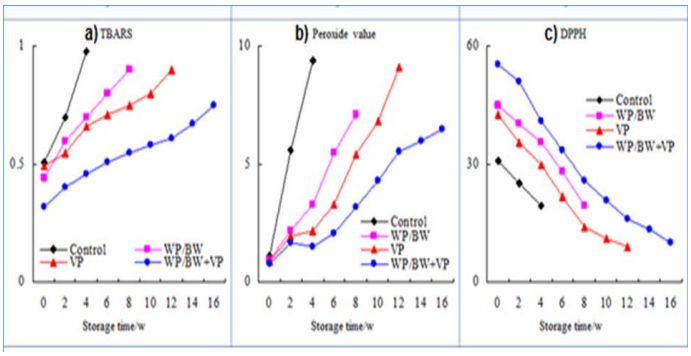

Figure 4: a) TBARS (mg malonaldehyde/kg), b) peroxide value (meq/kg fat) and c) free radical scavenging activity (DPPH) (g/100g) of sliced pastirma stored at 5°C for 16 weeks

Color evaluation

The lipid oxidation criteria were correlated with the changes in instrumental color indexes and the development of the brown color. The met-myoglobin content in all groups was below 1% and markedly increased during refrigeration storage suggesting the rapid oxidation of myoglobin (Fig. 5 a). However, the met-myoglobin percentage was the highest in vacuum packaged pastirma and the lowest in vacuum

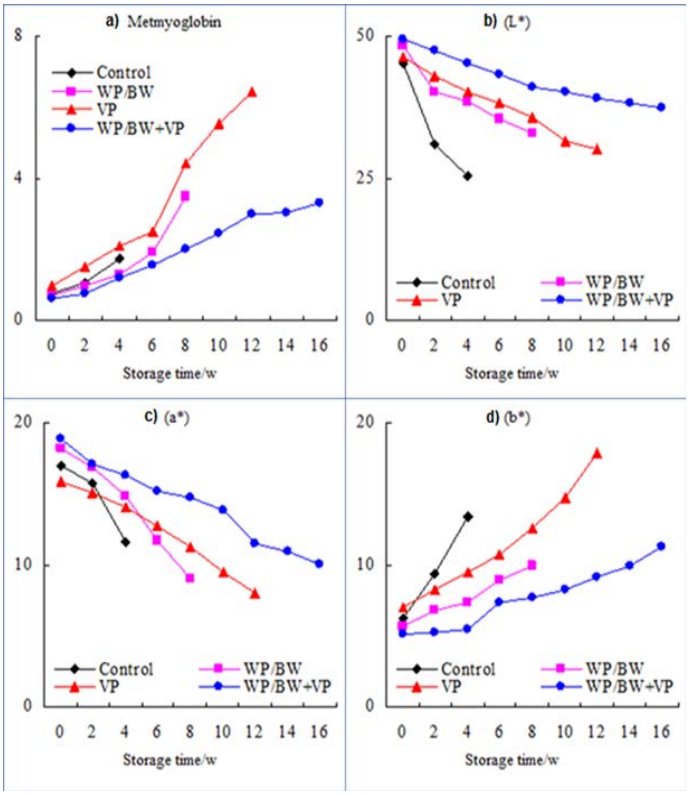

Figure 5: a) Met-myoglobin content (g/100g) and color indexes {b) L*, c) a* and d) b*} of sliced pastirma stored at 5°C for 16 weeks

packed WPI-coated samples. The obtained results were in agreement with those of Danijela et al. (2013) who suggested that vacuum packaging containing O_2 encourages myoglobin oxidation, while WPI edible coating reduced the autoxidation of myoglobin due to its antioxidant properties and low oxygen permeability (Song et al., 2020). The instrumental color evaluation follows the same trend of met-myoglobin formation, where the WPI coating increased (*L**) and (*a**) while decreased (*b**) values shortly after application compared to control and vacuum-packed sliced pastirma (Fig. 5 b, c, d). Higher *(L*)* value of coated pastirma may be related to the high barrier ability of WPI/BW coating (Song et al., 2020) which, prevent the loss of chemical component of pastirma either immediately after processing or during the chilling storage. Fischer (2007) reported that there was a proportional association between the *(L*)* value and the fat content of meat. Furthermore, elevation of (*a**) value and reduction of (*b**) value by the application of WPI/BW coating may be related to the antioxidant activity and gas barrier properties of the coat (Song et al., 2020) which, prevent the conversion of myoglobin (red color) to met-myoglobin (brown color) leading to preservation of the product's color either immediately after processing or during the chilling storage. On the other hand (*L**) and (*a**) values showed a steady decrease while the (*b**) increased in all treatments during refrigerated storage. The changes in color in control and vacuum-packed samples were more evident than WP coated samples and the difference in vacuum packaged pastirma was less than aerobically packaged ones which indicates the beneficial effect of combined WP coating and vacuum packing in minimizing color oxidation.

Lipid oxidation is a good indicator for sensory acceptability because the oxidation radicles are additionally formed secondary radicles that are closely associated with deteriorative changes especially myoglobin autoxidation (Wang et al., 2021). Therefore, the safety, shelf life, and acceptability of meat products can be determined based on the changes in color because once the color is judged unacceptable, all other sensory attributes lose their significance to the consumers.

CONCLUSION

It could be concluded that the application of WPI/BW-edible coating and vacuum packaging improved the oxidation stability indexes and increased the shelf life of sliced pastirma as a consequence of the reduction in bacterial loads, TBARS, PV, carbonyl compound, pH value, and met-myoglobin content with an increase in the scavenging activity of such treatment.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors pronounce that they have no conflict of interest.

NOVELTY STATEMENT

Application of whey protein isolate/beeswax edible coating led to extension of the shelf life of sliced pastirma with maintaining its safety and quality, where in this study the coated sliced pastirma either aerobically or vacuum packed had storage life of 16 and 8 weeks at 5°C, respectively in comparison to 4 weeks for uncoated pastirma.

AUTHORS CONTRIBUTION

Each author contributed in these research by 25%

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