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# Utilisation of pullulan active packaging incorporated with curcumin and pullulan mediated silver nanoparticles to maintain the quality and shelf life of broiler meat

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## ABSTRACT

Broiler meat can provide an appropriate environment for the microbial intensification, resulting in the lipid and protein oxidation and physico-chemical degradation. The utilisation of the anti-oxidants prevents meat degradation and human health challenges by preventing meat physico-chemical characteristics. Pullulan active packaging incorporated with green silver nanoparticles, as anti-oxidants, has not been reported in broiler meat preservation. Therefore, the impact of pullulan active packaging, incorporated with curcumin and pullulan mediated silver nanoparticles (C-AgNPs, P-AgNPs) on broiler meat was determined in the current study for 14 days of refrigerated storage at  $4 \pm 1^\circ\text{C}$ . A total of 120 broiler birds were reared for clean meat production and divided into 4 treatment groups (PF-CTRL, PF-C-AgNPs, PF-P-AgNPs, PF-M-AgNPs). Pullulan active packaging maintained meat textural attributes and pigment stability and breast muscle pH (6.11). Broiler meat treated with pullulan active packaging incorporated with curcumin arbitrated AgNPs (PF-C-AgNPs) indicated significantly higher oxidative stability ( $p = .000$ ), water holding capacity ( $p = .002$ ), expressible fluids ( $p = .000$ ), and shear force values ( $p = .000$ ) along with significantly lower cooking losses ( $p = .000$ ), pH ( $p = .000$ ), and shear force energy ( $p = .000$ ) as compared to the control (CTRL) and other treatment groups. Broiler breast meat samples treated with pullulan active packaging expressed a “lighter” appearance ( $53.61 \pm 3.64$ ) during the refrigerated storage of 14 days. Moreover, significant differences were recorded in the oxidative stability/MDA (mg/kg) concentrations amongst the controlled and the treated groups on day 7th ( $p = .000$ ) and day 14th ( $p = .000$ ) of refrigerated storage. The current study confirms that the pullulan active packaging, incorporated with curcumin arbitrated silver nanoparticles (PF-C-AgNPs) followed by pullulan active meat packaging incorporated with pullulan mediated silver nanoparticles (PF-P-AgNPs) maintained the textural and physico-chemical meat attributes along with minimal oxidative rancidity in 14 days of storage. It is concluded that PF-C-AgNPs and PF-P-AgNPs can be a safer antioxidant alternate to preserve broiler meat quality and oxidative stability with a prolonged shelf life.

## HIGHLIGHTS

- Pullulan active meat packaging, incorporated with C-AgNPs and P-AgNPs, should be considered as green and bio-degradable substitution of the hazardous synthetic antioxidant packaging.
- Pullulan active meat packaging (PF-C-AgNPs, PF-P-AgNPs) is able to maintain the textural characteristics, compositional integrity, and physico-chemical attributes of broiler meat.
- Broiler meat treated with PF-C-AgNPs and PF-P-AgNPs reflects better oxidative stability with prolonged shelf life.

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## KEYWORDS

Curcumin; free radicals scavenging; meat preservation; oxidative rancidity; pullulan active packaging

## Introduction

Poultry industry is one of the rapid growing and most proverbial sector providing high quality meat with

superior proteins, minerals, polyunsaturated fatty acid (especially omega 3-fatty acids) and vitamins (FAO 2016; Dalle Zotte et al. 2017; Rouger et al. 2017). Due

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to cheaper sources of protein, better digestibility and ease of cooking, the demands for broiler meat are increasing and it is projected more than 1.8 metric tons annually by the year 2025 (FAO 2016; Catarino et al. 2017). The broiler meat can be, easily, swarmed with various microbes due to its chemical profile providing a suitable growth environment resulting in the spoilage of meat (Dave and Ghaly 2011; Souza and Fernando 2016).

Lipid oxidation or oxidative rancidity in broiler meat is considered as one of the prime indicators of the meat quality (Sultana et al. 2017). The polyunsaturated fatty acid profile, presence of free iron and lack of any suitable anti-oxidant induce lipid oxidation with the formation of hazardous compounds like hydroperoxides, ketones, aldehydes and melanodialdehydes in the meat (Dave and Ghaly 2011; Liu et al. 2015; Sultana et al. 2017; Djenane and Roncalés 2018). To prevent these changes in meat, the use of synthetic antioxidants including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl-hydroquinone (TBHQ), propyl gallate (PG); nanoparticles (silver, gold, zinc); and natural substances like plant essential oils is in practice (Wu et al. 2013; Maqsood et al. 2015; Sultana et al. 2017). The emerging issues of meat borne illnesses, higher concentrations of antioxidants, and reduced meat quality demand for the development of environment friendly, safer and convenient technologies for prolonging the shelf life of food and meat (Chouhan et al. 2017; Gonelimali et al. 2018).

Pullulan is an extra cellular polysaccharide produced by a fungal species *Aureobasidium pullulans* and has been utilised as a 'biopolymer' for the synthesis of edible films and active packaging with an enhanced mechanical property and cohesive ability to the meat surface for the reduction of meat oxidative rancidity (Diab et al. 2001; Trinetta et al. 2011; Trinetta and Cutter 2016; Xiao et al. 2018; Khan et al. 2020). Green synthesised silver nanoparticle (AgNPs), as the active ingredient, are being utilised by meat and food industries and biomedical, and veterinary sectors (Khan, Kumari, et al. 2019). Lower chances of toxicity due to surface mediation, efficient release from human body (with crystalline size lower than 20 nm), and excellent antioxidant performance in polysaccharide active packaging make AgNPs as a better choice by food industry (Trinetta and Cutter 2016; Khan et al. 2020; Hussenen et al. 2021). The green synthesised pullulan mediated AgNPs (P-AgNPs) have an improved ability, as an active substance, when incorporated into pullulan edible films with more absorption and release mechanism to the meat surface by their minute size

and spherical shape (Khan, Shameli, et al. 2019). Curcumin, an active substance obtained from *Curcuma longa* tubers, has been historically used as an anti-oxidant, anti-inflammatory, and anti-cancer agent in the food and biomedical enterprises (Varaprasad et al. 2011; Shameli et al. 2014). Curcumin arbitrated AgNPs (C-AgNPs) can be considered as green, safer and cheaper antioxidant active substances for the preservation of meat due to their inimitable characteristics found in our previous study (Khan, Shameli, et al. 2019). The utilisation of green AgNPs, as antioxidants in active packaging, can minimise the risks of oxidative rancidity in meat during the storage and processing (Khalaf et al. 2013; Sultana et al. 2017).

The current study was, therefore, planned to detect the anti-oxidant capabilities of bio-degradable and environment friendly pullulan active meat packaging to maintain the quality of broiler meat during storage. Green P-AgNPs and C-AgNPs were incorporated into pullulan edible packaging as the 'active anti-oxidant substances' and the anti-oxidant potential of pullulan active meat packaging was explored at  $4 \pm 1$  °C (from 0 day to 14 days).

## Materials and methods

### Materials

2,2-Diphenyl-1-picrylhydrazyl (DPPH); 2-thiobarbituric acid (GR Grade); butylated hydroxytoluene ( $\geq 99\%$  pure); sodium dodecyl sulphate; 1,1,3,3-tetraethoxypropane; potassium chloride; ethanol (95% pure); 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS); methanol ( $\geq 99\%$  pure) and deionised water (DW) were purchased from Sigma-Aldrich (St. Louis, MO, USA) whereas potassium peroxodisulfate (potassium persulphate) and glacial acetic acid (99.9%) were obtained from HmBG Co. Inc. (Hamburg, Germany).

### Clean broiler breast meat (*Pectoralis major*) production

The broiler breast meat was produced following the animal ethics and protocols approved by 'Institutional Animal Care and Use Committee' of the University Putra Malaysia, Malaysia (UPM/IACUC/AUP-R088/2018) at experimental poultry farm. A total of one hundred and twenty (120) male day old broiler chicks (Cobb 500), with an average body weight of 42.34 g, were obtained from a local hatchery and were reared in the individual floor pens adopting 'deep litter system' with an *ad-libitum* feeding of commercial broiler feed (starter, grower, finisher; Table 1) and *ad-libitum*

**Table 1.** Nutrient profile of commercial broiler feed used in the rearing of the broilers.

Nutrient profile	Starter	Grower	Finisher
Crude protein, % (based on feed basis)	21.0	19.0	17.5
Crude fibre, %	5.0	5.0	6.0
Crude fat, %	4.5	5.0	4.0
Moisture contents, %	13.0	13.0	13.0
Metabolizable energy, Kcal/kg	3030	3060	3100
Ash, %	8.0	8.0	6.0
Calcium, %	0.8	0.8	0.8
Phosphorus, %	0.4	0.4	0.4

amount of drinking water during 35 days of rearing. Afterwards, the birds were kept on 6 h of rest (lairage) period with continuous clean water supply before slaughtering. At the age of 36th day, the birds, with an average body weight of more than 2.0 kg, were humanely slaughtered through 'Halal procedure' as described on the MS 1500: 2009 (Department of Standards Malaysia 2009). After slaughtering, dressing, evisceration and elimination of head, wings and claws from bird's carcasses, the breast meat was trimmed off of all visible fats.

### Application of pullulan active packaging on broiler breast meat

Pullulan active packaging was formulated as per procedure reported by Khalaf et al. (2013) and Morsy et al. (2014) with a modification by adding 2% (w/v) of silver nanoparticles (AgNPs). Four treatment groups were generated randomly [1 = treated with pullulan edible film (PF-CTRL), 2 = treated with pullulan edible film incorporated with curcumin silver nanoparticles (PF-C-AgNPs), 3 = treated with pullulan edible film incorporated with pullulan mediated silver nanoparticles (PF-P-AgNPs), and 4 = treated with pullulan edible film incorporated with mixed silver nanoparticles (PF-M-AgNPs)] with three (0 day, 7 days and 14 days) storage durations and thirty (30) birds in each treatment (Stutely 2003; Faul et al. 2007). The meat samples ( $k$ ) for CTRL were taken from all the groups by using 'systematic random sampling' (Taherdoost 2016; Hayes 2018) as per the given equation:

$$\text{Random number } (k) = N/n, \quad (1)$$

where  $k$  = random number of the meat sample,  $N$  = total number of meat samples, and  $n$  = required number of meat sample.

The right *Pectoralis major* muscle was removed, cut into pieces at a dimension of 5 cm × 3 cm × 2 cm (length × width × depth), packed into the polyethylene zip-locker bags, wrapped into aluminium foil and stored at -80 °C before treatment (Cantalejo et al. 2016). The rectangular strips of pullulan active packaging (4 cm ×

2 cm and 4 cm × 1 cm) were applied on the meat samples as a bi-layer coating (Figure 1) for different physico-chemical analysis with vacuum packaging. The vacuum-packed meat samples were stored at 4 ± 1 °C for 7 and 14 days. Various parts of the meat samples, obtained from right side of broiler breast (*Pectoralis major*), were used at 0 day, 7th day and 14th day of storage for the physio-chemical analyses as shown in Figure 1.

### Broiler breast meat quality assessment

#### pH of broiler breast (*Pectoralis major*) meat:

The pH values of the meat samples ( $n = 10$ ) were measured according to the methodology presented by Zhang et al. (2016) with slight modifications.

#### Colour determination

For the colour assessment, the meat samples, with a dimension of 3 cm × 2 cm × 1 cm (length × width × depth), were obtained from broiler breast meat, wrapped into pullulan active packaging and stored at 4 ± 1 °C in vacuum packaging for 0, 7, and 14 days. After storage, the packaging was removed and three observations per samples were recorded at three random points according to the procedure of Noori et al. (2018). The lightness ( $L^*$ ), yellowness ( $a^*$ ), redness ( $b^*$ ), chromatography ( $\Delta E$ ), chroma ( $C$ ) and hue ( $H$ ) were calculated. The meat samples at 0 day of storage (from the CTRL group) were used as 'standard ( $s$ )'. The equations to calculate chromatography ( $\Delta E$ ), chroma ( $C$ ), and hue ( $H$ ) are as below

$$\begin{aligned} \text{Chromatography } (\Delta E) &= \sqrt{(L^* - L^s)^2 + (a^* - a^s)^2} \\ &\quad + (b^* - b^s)^2, \end{aligned} \quad (2)$$

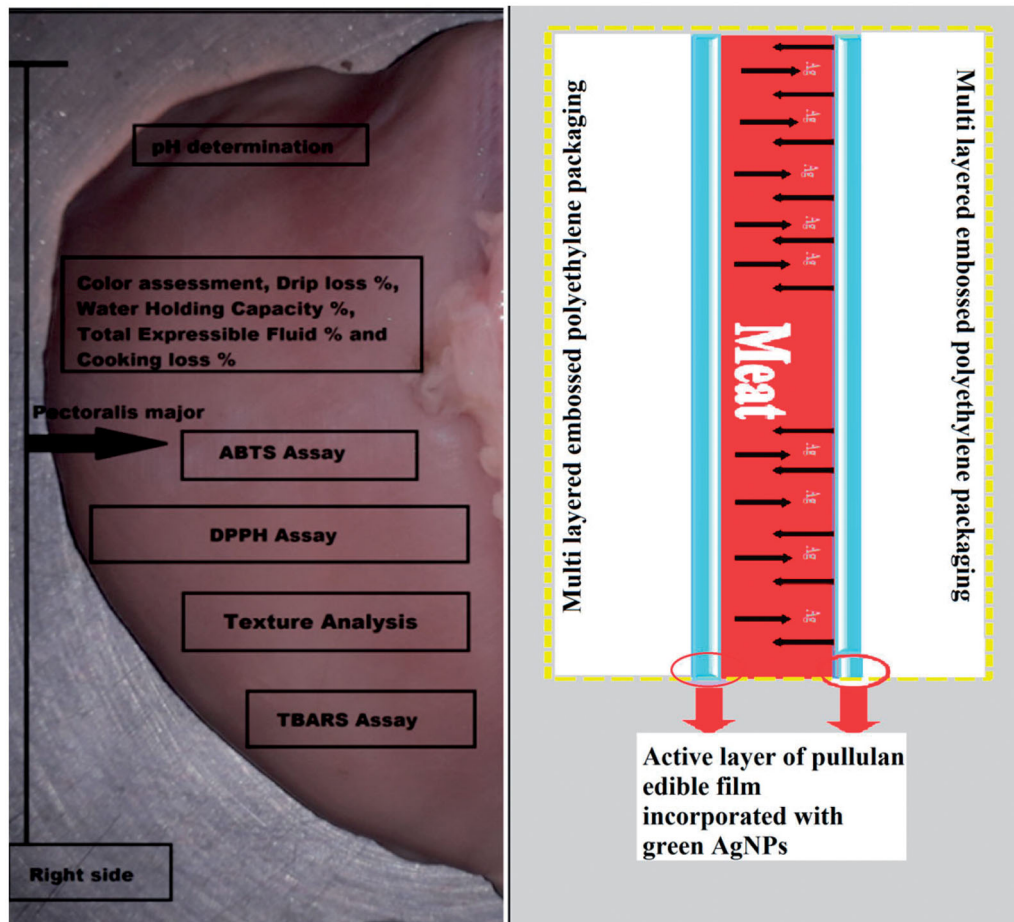
where  $L^s$ ,  $a^s$ , and  $b^s$  represent lightness, yellowness and redness of standard meat samples from control negative (CTRL) group at day 0 of storage.

$$\text{Chroma } (C) = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

$$\text{Hue } (H) = \tan^{-1}(b^*/a^*) \quad (4)$$

#### Drip loss percentage

Drip loss percentages (DL %) were calculated on 7th and 14th day of refrigerated storage of meat samples according to the methodology reported by Yang et al. (2018). Meat samples, with a dimension of 2 × 2 × 1 cm<sup>3</sup>, were cut from each treatment group after 24 h post-mortem. Ten meat samples ( $n = 10$ ) were obtained from the treatment groups wrapped into pullulan edible film, except control negative (CTRL),



**Figure 1.** Application of pullulan active packaging on broiler breast meat ( $n = 120$ ) along with the presentation of meat areas used in various physio-chemical analyses. [ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl ; AgNPs: silver nanoparticles; TBARS: thiobarbituric acid reactive substances].

and were vacuum packed using chamber vacuum sealer VS-168 (QuiWARE, KL, Malaysia) into a multi-layered embossed polyethylene bag supported with an outer layer of nylon (PA+PE 2, 180  $\mu$  thickness, 20  $\times$  30; QuiWARE, KL, Malaysia). The packed meat samples were placed in refrigerated storage at  $4 \pm 1$   $^{\circ}$ C for 7 and 14 days simultaneously. After storage (at 7th and 14th days) the meat samples were taken out from the polyethylene bags and placed on a double layer of filter paper (grade 4, 20–25  $\mu$ m; Whatman<sup>®</sup>) under the load of 20 kg for 6 h. Initial weight ( $W_i$ ) and final weights ( $W_f$ ) were recorded and water holding capacity was calculated by the formula given as under:

$$\text{Drip loss percentage (DL \%)} = [100 \times (w_d/w_i)]. \quad (5)$$

#### **Water holding capacity and expressible fluid percentages**

Water holding capacity of meat samples was calculated after 7th and 14th days of refrigerated storage as per

methodology provided by Carvalho et al. (2017). Initial weight ( $W_i$ ) and final weight ( $W_f$ ) were recorded and water holding capacity was calculated by the formula as below:

$$\begin{aligned} \text{Water holding capacity (WHC \%)} \\ = 100 - [100 \times (W_i - W_f)/W_i]. \end{aligned} \quad (6)$$

Similarly, the expressible fluid percentages were measured at 7th and 14th days of refrigerated storage as per the procedure reported by Yang et al. (2018).

The expressible fluid percentage (TEF %) was calculated by the following formula:

$$\begin{aligned} \text{Expressible fluid percentage (TEF \%)} \\ = [100 \times (A_f/A_w)]. \end{aligned} \quad (7)$$

#### **Cooking loss and texture analysis of broiler breast (Pectoralis major) meat**

For the estimation of cooking loss percentages, ten meat samples ( $n = 10$ ), with a dimension of 2 cm  $\times$  1 cm  $\times$  1 cm (length  $\times$  width  $\times$  depth) were obtained

from each treatment group, wrapped into pullulan active packaging, vacuumed packed and stored at  $4 \pm 1^\circ\text{C}$ . On day 0 (after 2 h of packing), 7 and 14, the cooking loss was calculated as per the methodology of Schwarz et al. (2019) the following equation;

$$\text{Cooking loss percentage (CL \%)} = (W_i - W_f) / W_i \times 100. \quad (8)$$

The meat samples were stored at  $3 \pm 1^\circ\text{C}$  for 24 h afterwards for texture analysis. The texture was analysed by the softness or gumminess of the meat samples assessed by peak shear force (g) using a texture analyser. The shear energy ( $\text{N} \times \text{mm}$ ) of meat samples was also calculated (Bowker and Zhuang 2019). 'Meat softness/hardness or gumminess' was calculated by the shear force (g) and shear energy ( $\text{N} \times \text{mm}$ ) reflected the 'chewiness' of the meat samples (Soglia et al. 2016). Ten meat samples ( $n = 10$ ) were used per treatment and the readings were taken in triplicate at random points of each sample.

#### **Determination of oxidation stability of broiler breast (*Pectoralis major*) meat**

Lipid per-oxidation of the meat samples was premeditated according to the procedure adopted from Ramiah et al. (2014). At 0, 7th and 14th day of storage, a 1 g meat sample, was homogenised in 4 mL of 1.15% KCL aqueous solution at 20,000 rpm for 2 min using a tissue homogeniser TH-115 V (Omni Inter. CA, USA). After 10 min of incubation at  $24\text{--}25^\circ\text{C}$ , 200  $\mu\text{L}$  homogenised sample + 300  $\mu\text{L}$  de-ionized water + 35  $\mu\text{L}$  of 7 mM BHT solution + 165  $\mu\text{L}$  of 8.1% aqueous solution of SDS + 2 mL of 0.8% TBA solution were mixed into a 5 mL eppendorf tube. The mixed solution was heated at  $95 \pm 1^\circ\text{C}$  using a water bath WNB-14 (Memmert GmbH, Schwabach, Germany) for 60 min. The mixture was then cooled by using tap water and incubated at  $24\text{--}25^\circ\text{C}$  for 20 min and centrifuged at 1000 xg for 10 min at  $25^\circ\text{C}$  using a refrigerating micro centrifuge 5810 R (Sigma-Aldrich, Inc., St. Louis, USA). The supernatant was separated and absorbance was checked by a UV-VIS spectrophotometer UV-1800 (SHIMADZU Corp., Kyoto, Japan) at 532 nm wavelength against a blank without sample. The 'melanodialdehydes' concentration (mg MDA/kg of meat), measured from a standard curve of 1,1,3,3-tetraethoxypropane, were expressed as 'thiobarbituric acid reactive substances (TBARS) values.

2,2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays of meat samples were conducted according to the procedure of Sultana et al. (2017). A  $1 \pm 0.05$  g of meat sample was taken from the meat obtained from

all the treatment groups, wrapped into the rectangular strips ( $4 \times 1 \text{ cm}^2$ ) of pullulan active packaging, vacuum packed and stored for 0, 7, and 14 days. After storage, the meat samples were taken out from the packaging and 250 mg of the sample + 5 mL of ethanol (95%) were homogenised at 20,000 xg for 5 min using a tissue homogeniser TH-115 V (Omni Inter. CA, USA). The homogenised samples were centrifuged at 1200 rpm at  $4^\circ\text{C}$  for 40 min using a refrigerating micro centrifuge 5810 R (Sigma-Aldrich, Inc., St. Louis, USA). The supernatant was filtered by a filter paper (grade 1, 11  $\mu\text{m}$ ,  $460 \times 570 \text{ mm}^2$ ; Whatman®) and used for the analysis. The free radicals were generated by using 5 mL of ABTS/DPPH solution (7 mM) and incubated into the dark for 16 h. After incubation, the assorted solution of ABTS/DPPH was adjusted to acquire an absorbance of  $0.7 \pm 0.05$  at 732 nm wavelength. Adjusted ABTS/DPPH solutions + sample filtrate was vortexed for 1-2 min into the dark, incubated at  $25^\circ\text{C}$  for 10 min in the dark, centrifuged at 2000 at  $4^\circ\text{C}$  for 10 min by a refrigerating micro centrifuge 5810 R (Sigma-Aldrich, Inc., St. Louis, USA) and incubated again at  $25^\circ\text{C}$  for 30 min in the dark. The absorbance of the samples was recorded for ABTS and DPPH scavenging activities at 734 and 517 nm wavelength respectively against a blank solution. Free radical scavenging activity was calculated by the following equations:

$$\begin{aligned} &\% \text{ Radical scavenging activity (ABTS)} \\ &= [(Abs^c - Abs^a) \times 100] / Abs^c \quad (9) \end{aligned}$$

where  $Abs^c$  = absorbance of the blank, and  $Abs^a$  = absorbance of the samples.

$$\begin{aligned} &\text{DPPH radical scavenging activity (\%)} \\ &= [1 - (Abs^a - Abs^e) / Abs^c] \times 100 \quad (10) \end{aligned}$$

where  $Abs^a$  = absorbance of the sample + DPPH solution,  $Abs^e$  = absorbance of the samples (without DPPH solution), and  $Abs^c$  = absorbance of DPPH solution.

#### **Statistical analysis**

A completely randomised design (CRD) was used for the current study and the results were expressed as mean  $\pm$  standard deviation. Pooled standard error of means ( $SEM_p$ ) was also determined for each variable. A total of ten random samples per treatment group ( $n = 10$ ) were used except for the colour assessment in which three random samples per treatment group ( $n = 3$ ) were analysed. The analysis of the data was conducted by 'one-way' ANOVA (Khalaf et al. 2013; Carvalho et al. 2017;

Sultana et al. 2017) using the SPSS software V. 20.0 (IBM Corp., Armonk, NY, USA). The statistical differences

Strength	Stronger	Strong	Moderate	Weak
r Values	0.8–0.99	0.6–0.79	0.4–0.59	0.2–0.39

between means ( $p < .05$ ) were identified by 'Tukey's multiple comparison' test. The correlation between the variables was calculated by using CurveExpert software V. 1.4 (Daniel Hyams, Hixson, TN, USA) with 'rational function fit' for WHC: DL percentages [ $Y = (2.083 + 2.847x) \div (1 + 3.151x + 9.467x^2)$ ] of broiler meat (Nakyinsige et al. 2015; Sultana et al. 2017).

The strength of correlation was expressed as below reported:

(Bowker and Zhuang 2019).

## Results

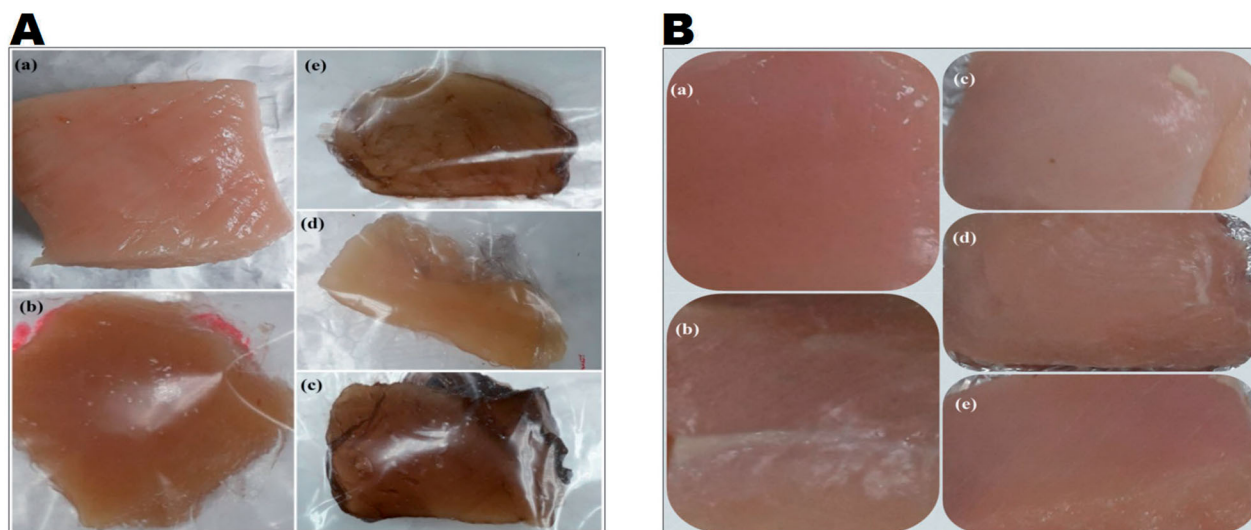
### Colour and appearance of broiler breast (*Pectoralis major*)meat

In the present study, the meat colour tended to become darker from day 0 (Figure 2(A)) to day 14th of storage (Figure 2(B)) whether it was treated with pullulan active meat packaging or not.

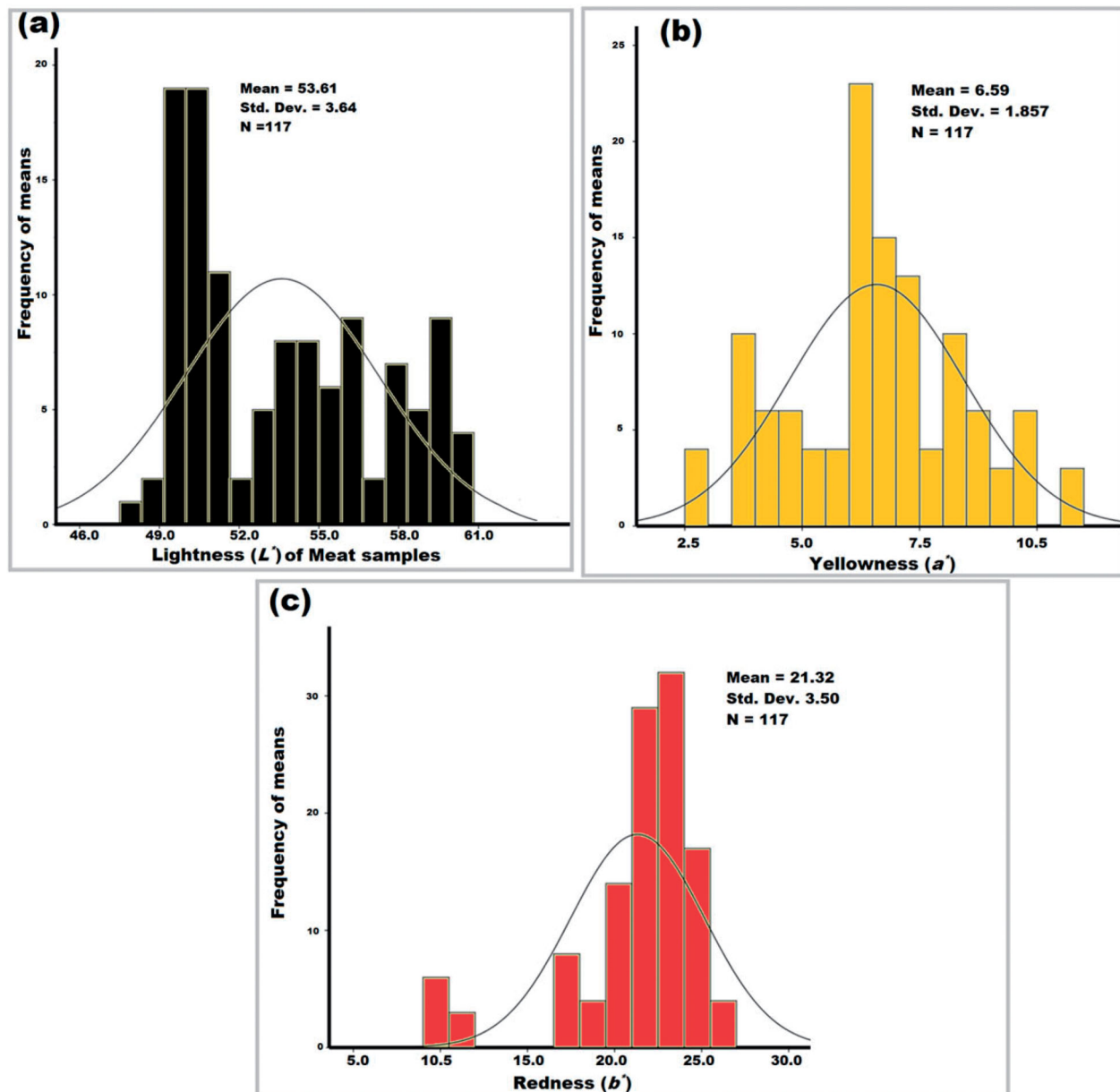
The lightness of broiler breast meat (*Pectoralis major*) was recorded as  $53.61 \pm 3.64$  (Figure 3(a)) with a 0.404 skewness and  $-1.20$  kurtosis and the meat can be categorized 'lighter' ( $L^* + \text{mean} > 50$ ; Petracci et al. 2004).

The correlation between the lighter meat and lower pH of broiler meat was also recorded in our study. The meat samples treated with PF-C-AgNPs, PF-P-AgNPs and PF-M-AgNPs exhibited significant differences ( $p < .05$ ) from the standard (CTRL) and the controlled meat samples (PF-CTRL) at day 0, day 7th and day 14th of the refrigerated storage (Table 2). A significant increase in lightness ( $L^*$ ) of broiler meat treated with PF-C-AgNPs active packaging was noticed at day 7th and was restored to the normal ( $L^* \leq 50$ ) at 14th day of storage.

In general, the average yellowness ( $a^*$ ) was recorded as  $6.59 \pm 1.857$  (Figure 3(b)) while the redness ( $b^*$ ) of broiler breast meat as  $21.32 \pm 3.50$  (Figure 3(c)). The broiler meat yellowness ( $a^*$ ) and redness ( $b^*$ ), treated with pullulan active packaging was recorded as significantly higher ( $p < .05$ ) as compared to the standard (CTRL) and the controlled (PF-CTRL) meat samples. The meat samples treated with PF-C-AgNPs, PF-P-AgNPs and PF-M-AgNPs active packaging reflected significantly higher ( $p < .05$ ) yellowness ( $a^*$ ) as compared to CTRL and PF-CTRL treated groups at day 0 and day 7th of the refrigerated storage. The meat samples treated with PF-C-AgNPs were noticed as significantly yellower ( $p < .05$ ) as compared to the other treatment groups (PF-CTRL, PF-P-AgNPs, PF-M-AgNPs) on day 14th of the storage. Furthermore, the redness ( $b^*$ ) of the meat samples treated with PF-C-AgNPs, PF-P-AgNPs and PF-M-AgNPs active packaging was recorded significantly



**Figure 2.** Expression of the variation in the colour of broiler breast (*Pectoralis major*) meat treated with pullulan active packaging; (A) at 0 day of refrigerated storage (a) CTRL (b) PF-CTRL (c) PF-C-AgNPs (d) PF-P-AgNPs and (e) PF-M-AgNPs. (B) at 14th day of refrigerated storage (a) CTRL (b) PF-CTRL (c) PF-C-AgNPs (d) PF-P-AgNPs and (e) PF-M-AgNPs [(a) CTRL: without packaging; (b) PF-CTRL: treated with pullulan edible film; (c) PF-C-AgNPs: treated with pullulan edible film incorporated with curcumin arbitrated; (d) PF-P-AgNPs: treated with pullulan edible film incorporated with pullulan mediated silver nanoparticles; (e) PF-M-AgNPs: treated with pullulan edible film incorporated with mixed silver nanoparticles].



**Figure 3.** Distribution of the colour attributes of broiler breast meat (*Pectoralis major*) treated with pullulan active packaging during 14 days of refrigerated storage (a) lightness ( $L^*$ ) (b) yellowness ( $a^*$ ) and (c) redness ( $b^*$ ).

elevated ( $p < .05$ ) as compared to CTRL and PF-CTRL treatment groups at day 0 and day 7th whereas the meat samples treated with PF-P-AgNPs and PF-P-AgNPs showed significantly higher ( $p < .05$ ) redness ( $b^*$ ) at day 14th (Table 2).

#### ***pH of broiler meat treated with pullulan active packaging***

Figure 4 provides a detailed portrait of pH of broiler meat samples treated with pullulan active packaging during 14 days of storage at  $4 \pm 1^\circ\text{C}$ . The pH of all the treated samples (PF-CTRL, PF-C-AgNPs, PF-P-AgNPs, PF-M-AgNPs)

was significantly lower ( $p < .05$ ) than that of the controlled group (CTRL; without packaging) whereas the pH of meat samples treated with PF-C-AgNPs active packaging was recorded as the lowest ( $5.80 \pm 0.13$ ) among all the treatment groups (Figure 4(a)). No significant differences ( $p < .05$ ) were recorded between the pH of meat samples at 7th ( $6.23 \pm 0.44$ ) and 14th day ( $6.26 \pm 0.54$ ) of refrigerated storage but were significantly higher ( $p < .05$ ) than the pH at day 0 ( $5.85 \pm 0.086$ ). Overall pH of all meat samples was recorded as  $6.11 \pm 0.445$  during 14 days of refrigerated storage (Figure 4(b)) and can be considered as 'safer' for human consumption without any foul odour (Lee et al. 1996; Dal et al. 2018).



**Table 2.** Colour attributes of broiler breast meat (*Pectoralis major*), treated with pullulan active packaging, during refrigerated storage.

Storage days	CTRL	PF-CTRL	PF-C-AgNPs	PF-P-AgNPs	PF-M-AgNPs	SEM <sub>p</sub>	p-Value
<b>Lightness (L*)</b>							
0	49.72 <sup>Bb</sup>	58.08 <sup>Aa</sup>	52.20 <sup>b</sup>	54.96 <sup>a</sup>	53.60 <sup>ab</sup>	0.57	.000
7th	49.72 <sup>Bb</sup>	51.61 <sup>Bb</sup>	59.57 <sup>a</sup>	51.89 <sup>Bb</sup>	52.86 <sup>b</sup>	0.56	.000
14th	49.72 <sup>ab</sup>	54.20 <sup>Aa</sup>	52.90 <sup>a</sup>	51.16 <sup>a</sup>	54.11 <sup>Aa</sup>	0.48	.006
<b>Yellowness (a*)</b>							
0	3.45 <sup>b</sup>	4.36 <sup>b</sup>	6.46 <sup>a</sup>	6.44 <sup>a</sup>	5.89 <sup>a</sup>	0.22	.000
7th	3.45 <sup>c</sup>	5.35 <sup>b</sup>	8.21 <sup>a</sup>	8.30 <sup>a</sup>	7.02 <sup>aa</sup>	0.31	.000
14th	3.45 <sup>c</sup>	8.26 <sup>a</sup>	6.41 <sup>Bb</sup>	8.81 <sup>a</sup>	6.64 <sup>ab</sup>	0.33	.000
<b>Redness (b*)</b>							
0	10.30 <sup>c</sup>	20.03 <sup>b</sup>	23.42 <sup>a</sup>	23.46 <sup>a</sup>	24.08 <sup>a</sup>	0.81	.000
7th	10.30 <sup>c</sup>	20.57 <sup>b</sup>	22.31 <sup>a</sup>	23.54 <sup>Aa</sup>	24.42 <sup>Aa</sup>	0.79	.000
14th	10.30 <sup>c</sup>	20.38 <sup>b</sup>	19.31 <sup>b</sup>	22.56 <sup>a</sup>	22.80 <sup>a</sup>	0.72	.000
<b>Meat chromatography (ΔE)</b>							
0	–	12.96 <sup>a</sup>	13.92 <sup>a</sup>	14.68 <sup>a</sup>	14.39 <sup>a</sup>	0.38	.424
7th	–	10.88 <sup>b</sup>	16.31 <sup>a</sup>	14.76 <sup>b</sup>	15.05 <sup>ab</sup>	0.39	.000
14th	–	12.59 <sup>ab</sup>	10.27 <sup>c</sup>	13.54 <sup>a</sup>	14.38 <sup>A</sup>	0.34	.000
<b>Chroma (C)</b>							
0	10.87 <sup>c</sup>	20.50 <sup>b</sup>	24.29 <sup>a</sup>	24.11 <sup>a</sup>	24.82 <sup>a</sup>	0.83	.000
7th	10.87 <sup>c</sup>	21.25 <sup>b</sup>	23.78 <sup>a</sup>	24.97 <sup>a</sup>	25.44 <sup>a</sup>	0.84	.000
14th	10.87 <sup>d</sup>	22.04 <sup>ab</sup>	20.37 <sup>c</sup>	24.16 <sup>A</sup>	23.79 <sup>a</sup>	0.76	.000
<b>Hue (H)</b>							
0	71.55 <sup>b</sup>	77.71 <sup>Aa</sup>	74.65 <sup>a</sup>	74.57 <sup>a</sup>	76.31 <sup>Aa</sup>	0.42	.000
7th	71.55 <sup>ab</sup>	75.57 <sup>Aa</sup>	69.86 <sup>b</sup>	70.59 <sup>b</sup>	73.94 <sup>a</sup>	0.46	.000
14th	71.55 <sup>ab</sup>	68.03 <sup>b</sup>	71.44 <sup>ab</sup>	68.67 <sup>b</sup>	73.98 <sup>a</sup>	0.55	.001

Means were compared at  $p < .05$ . The values with the same superscripts, within the same rows, are not significantly different. CTRL: without packaging; PF-CTRL: treated with pullulan edible film; PF-C-AgNPs: treated with pullulan edible film incorporated with curcumin arbitrated AgNPs; PF-P-AgNPs: treated with pullulan edible films incorporated with pullulan mediated AgNPs; PF-M-AgNPs: treated with pullulan edible film incorporated with mixed AgNPs. SEM<sub>p</sub>: Pooled standard error of means.

### Expressible fluid percentage

The results of the present study demonstrated that the total expressible fluid percentage (TEF %) of the meat samples treated pullulan active packaging was significantly higher ( $p < .05$ ) than the meat samples without packaging (CTRL) at 7th and 14th days of refrigerated storage (Figure 5(a)). It was, further, found that the meat samples treated with PF-C-AgNPs active packaging exhibited significantly higher ( $p < .05$ ) TEF % amongst all the treatment groups followed by PF-P-AgNPs, PF-M-AgNPs and PF-CTRL treatments (Table 3).

### Drip loss percentage

The drip loss (DL) percentages of the meat samples treated with PF-C-AgNPs and PF-M-AgNPs active packaging were significantly lower ( $p < .05$ ) than CTRL (without packaging), PF-CTRL (without AgNPs) and PF-P-AgNPs treatment groups (Figure 5(d)). The drip losses at 7th day ( $2 \pm 1.29$ ) were significantly lower ( $p < .05$ ) than those at 14th day ( $4.25 \pm 1.7$ ) of refrigerated storage (Figure 5(d)).

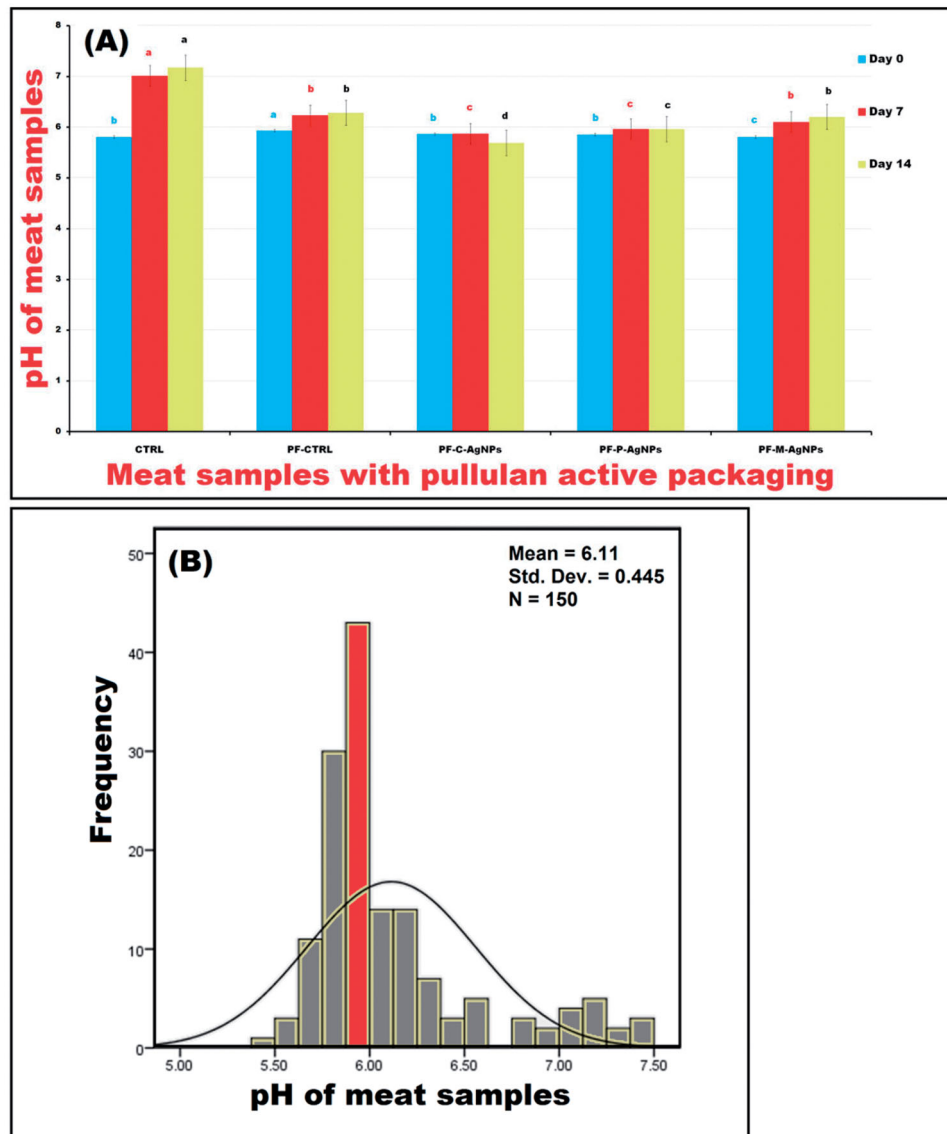
### Water holding capacity of broiler meat

In the current study, the meat samples treated with pullulan active packaging exhibited statistically ( $p < .05$ )

better WHC than CTRL (without packaging) with an overall percentage of  $82.82 \pm 5.274$  (Figure 5(c)). It was noticed that the meat samples treated with PF-C-AgNPs represented higher WHC percentage amongst all the treatment groups followed by PF-M-AgNPs, PF-P-AgNPs, and PF-CTRL (Figure 5(b)). The WHC percentages were significantly reduced ( $p < .05$ ) with the advancement of the refrigerated storage from day 7 ( $87.22 \pm 2.05$ ) to day 14 ( $78.42 \pm 3.54$ ). A strong and negative correlation between WHC and drip loss percentages ( $r = 0.94057843$ ; Figure 5(e)) is also reported in this study.

### Texture analysis

No significant differences were recorded in the softness of meat at day 0 of refrigerated storage with an overall mean value of  $349.43 \text{ g} \pm 201.35$  (Figure 6(a)) while the meat samples without pullulan packaging (CTRL) exhibited significantly lower ( $p < .05$ ) softness/gumminess at 7th ( $693.67 \text{ g} \pm 300.88$ ) and 14th ( $772.97 \text{ g} \pm 499.07$ ) days of storage as compared to the meat samples treated with pullulan packaging (PF-CTRL) and PF-C-AgNPs, PF-P-AgNPs, and PF-M-AgNPs active packaging. It was also found that the meat samples, treated with PF-C-AgNPs active packaging, were significantly softer and tender ( $p < .05$ ) amongst all the treated groups at 7th ( $228.25 \text{ g} \pm 207.49$ ) and 14th ( $329.53 \text{ g} \pm 190.1$ ) days of refrigerated storage (Figure 6(b,c)) with a significantly lower ( $p < .05$ ) shear force



**Figure 4.** pH of broiler breast meat (*Pectoralis major*) treated with pullulan active packaging (A) at day 0, 7th, and 14th day of refrigerated storage and (B) an overall expression of pH.

(g) followed by PF-P-AgNPs, PF-M-AgNPs and PF-CTRL respectively (Table 3). Moreover, the chewiness of the meat samples treated with PF-C-AgNPs was better, with significantly lower ( $p < .05$ ) shear energy ( $N \times mm$ ) as compared to the other treated samples. The meat samples from CTRL and PF-CTRL treatments showed higher shear force values and lower chewiness (Table 3).

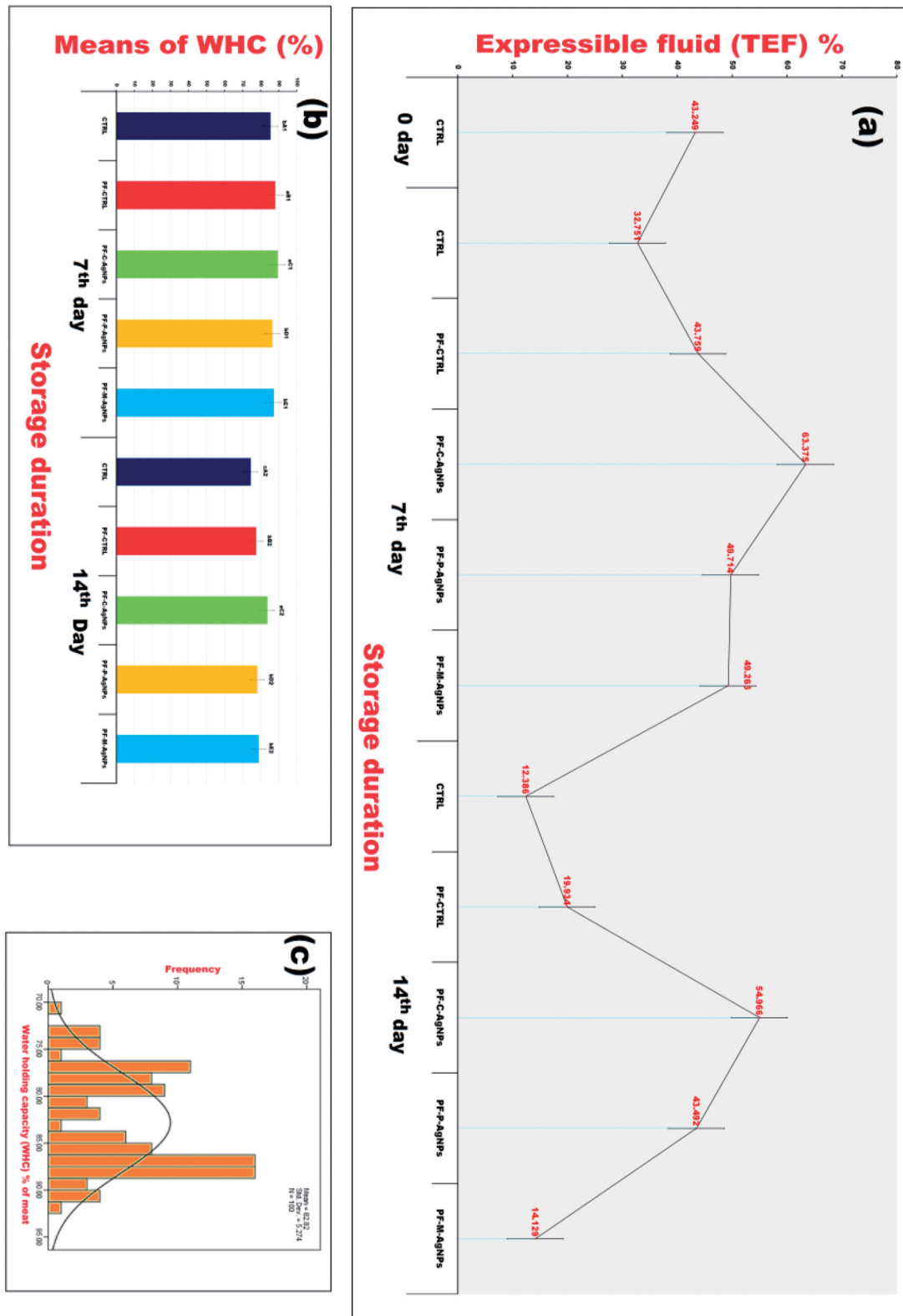
#### Cooking loss percentage

In this study, the cumulative cooking loss percentage was recorded as  $20.66 \pm 3.11$  (Figure 6(d)) and statistically lower ( $p < .05$ ) at day 7 ( $19.31 \pm 2.38$ ) as compared to day 0 ( $20.65 \pm 3.25$ ) and day 14 ( $20.02 \pm 3.06$ ) of the refrigerated storage. No significant differences ( $p <$

$.05$ ) were recorded in the cooking loss at day 0 whereas the treatment groups (PF-CTRL, PF-C-AgNPs, PF-P-AgNPs and PF-M-AgNPs) reflected the lower cooking losses as compared to CTRL group at day 0, 7 and 14 (Table 3).

#### Thiobarbituric acid reactive substance (TBARS) in broiler meat

For the determination of lipid peroxidation, it was detected that the oxidative stability in broiler meat was significantly reduced ( $p < .05$ ) with the increase in the storage duration (Figure 7(a,c)) with an overall MDA concentration as  $5.92 \pm 3.05$  mg/kg of meat (Figure 7(b)). Moreover, the meat samples treated with PF-C-AgNPs active packaging exhibited significantly the



**Figure 5.** Water retention in broiler breast meat (*Pectoralis major*) treated with pullulan active packaging (a) Expressible fluid percentage (b) water holding capacity (WHC %) of broiler breast meat (c) overall mean of WHC % of meat (d) drip loss percentages and (e) negative correlation of WHC and drip loss.

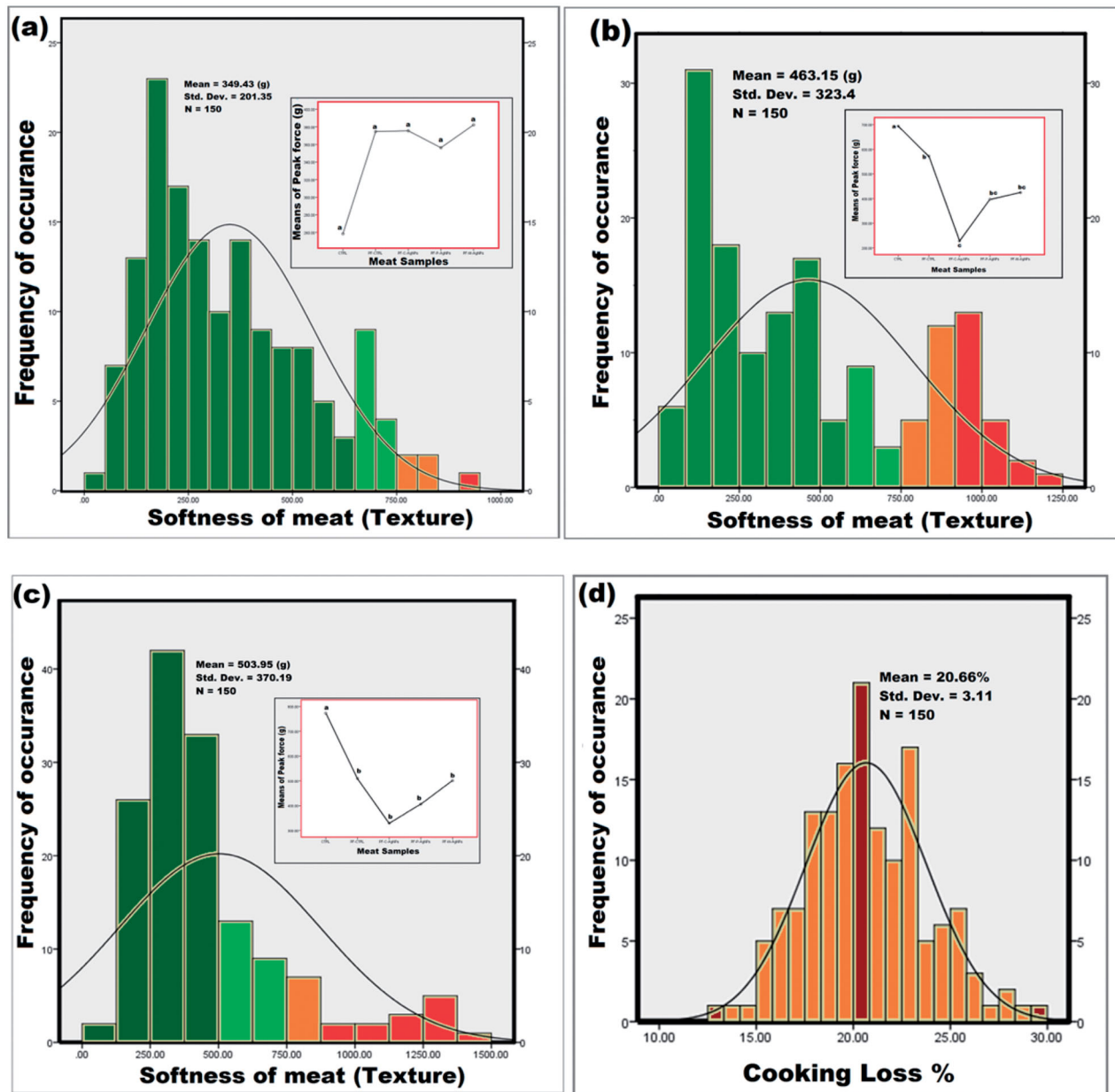
lowest ( $p < .05$ ) concentrations of MDA ( $3.29 \pm 1.84$  mg/kg of meat; Table 4) amongst all the treatment groups with enhanced oxidative stability.

The MDA concentrations were significantly affected ( $p < .05$ ) by the storage period as well with an amplified lipid per-oxidation on day 14th. A significantly

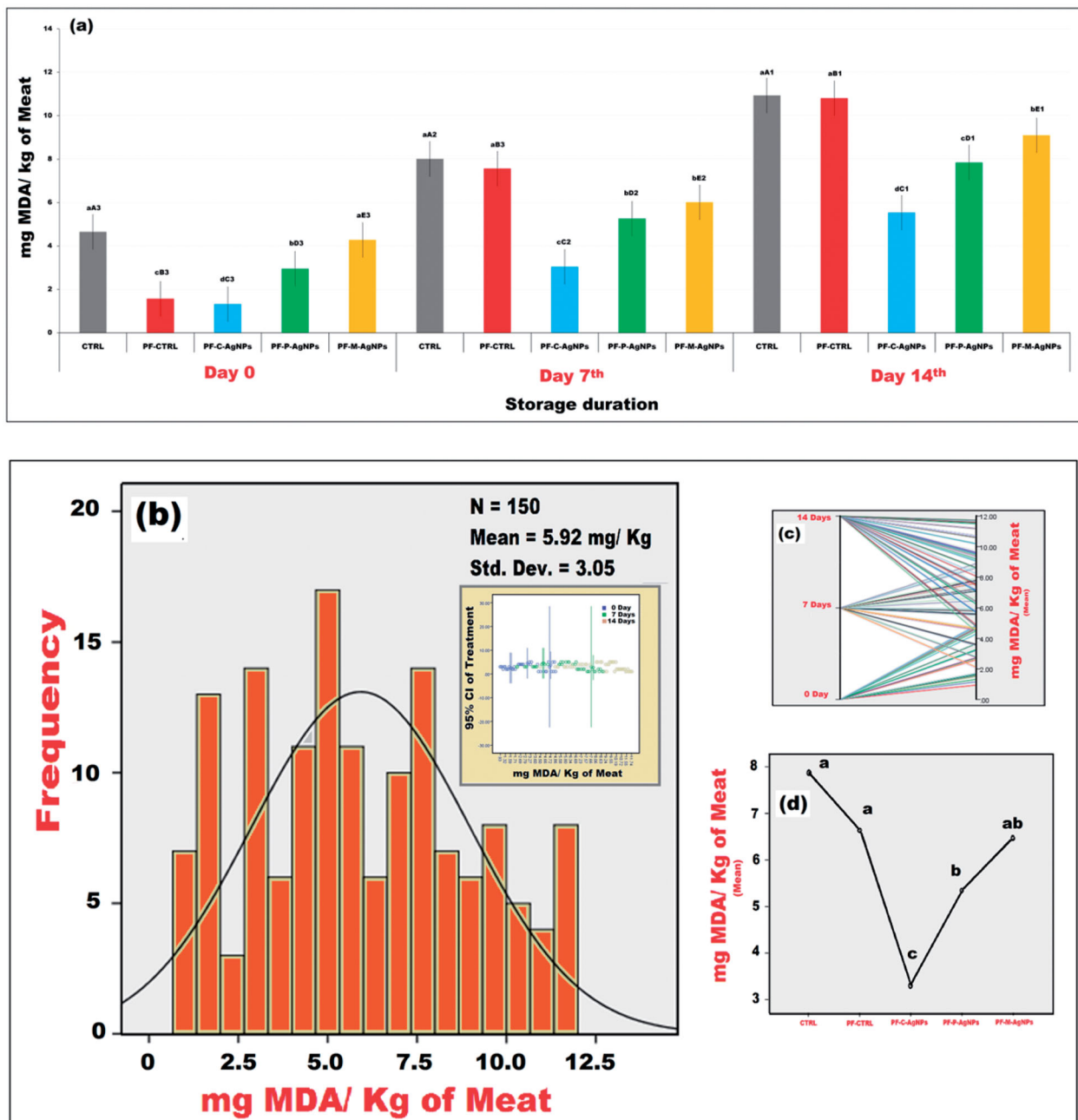
**Table 3.** Effect of pullulan active packaging on the physico-chemical characteristics of broiler breast meat (*Pectoralis major*).

	CTRL	PF-CTRL	PF-C-AgNPs	PF-P-AgNPs	PF-M-AgNPs	SEM <sub>p</sub>	p-Value
pH	6.66 <sup>a</sup>	6.15 <sup>b</sup>	5.80 <sup>c</sup>	5.92 <sup>b</sup>	6.02 <sup>b</sup>	0.04	.000
Expressible fluids, TEF, %	22.57 <sup>c</sup>	31.85 <sup>c</sup>	59.17 <sup>a</sup>	46.60 <sup>b</sup>	31.70 <sup>c</sup>	0.82	.000
Drip loss, %	4.46 <sup>a</sup>	2.95 <sup>b</sup>	1.69 <sup>b</sup>	4.24 <sup>ab</sup>	2.30 <sup>b</sup>	0.18	.000
Water holding capacity, %	79.92 <sup>b</sup>	82.64 <sup>ab</sup>	86.48 <sup>a</sup>	82.17 <sup>ab</sup>	82.88 <sup>ab</sup>	0.52	.002
Hardness/texture, g	575 <sup>Aa</sup>	485.46 <sup>a</sup>	311.11 <sup>b</sup>	386.61 <sup>ab</sup>	436.0 <sup>a</sup>	–	.000
Shear energy, N × mm	5.64 <sup>Aa</sup>	4.76 <sup>a</sup>	3.05 <sup>b</sup>	3.79 <sup>ab</sup>	4.28 <sup>a</sup>	0.14	.000
Cooking loss, %	22.55 <sup>a</sup>	21.43 <sup>a</sup>	18.52 <sup>b</sup>	20.40 <sup>ab</sup>	20.44 <sup>ab</sup>	0.30	.000

Means were compared at  $p < .05$ . The values with the same superscripts, within the same rows, are not significantly different. CTRL: without packaging; PF-CTRL: treated with pullulan edible film; PF-C-AgNPs: treated with pullulan edible film incorporated with curcumin arbitrated AgNPs; PF-P-AgNPs: treated with pullulan edible films incorporated with pullulan mediated AgNPs; PF-M-AgNPs: treated with pullulan edible film incorporated with mixed AgNPs. SEM<sub>p</sub>: Pooled standard error of means. For Meat texture SEM<sub>p</sub> could not be calculated due to the impacts of storage duration, various groups, and variation in data.



**Figure 6.** Softness/tenderness of broiler breast meat (*Pectoralis major*) (a) at 0 day (b) at 7th day and (c) at 14th day of storage, and (d) cooking loss of me.

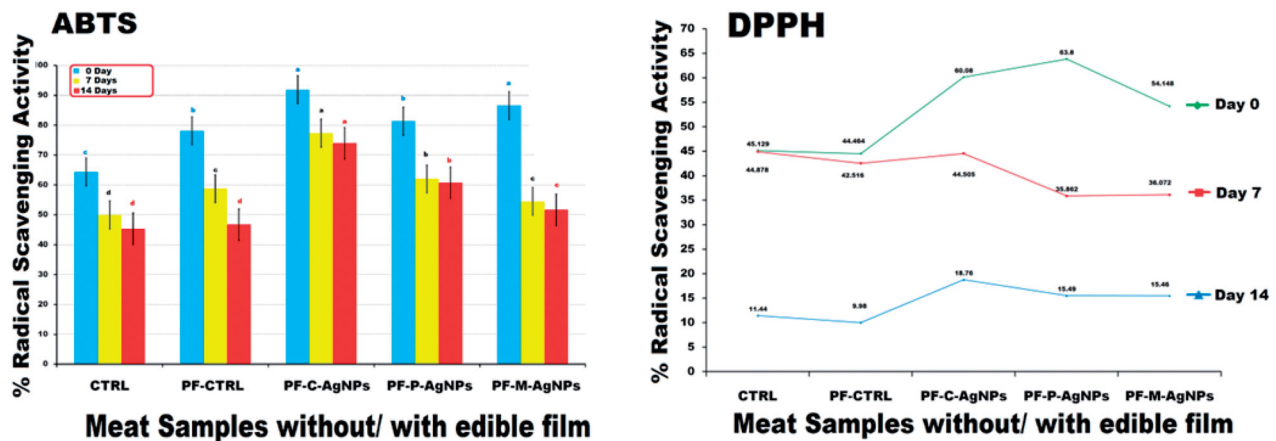


**Figure 7.** Oxidative stability of broiler breast meat (*Pectoralis major*) treated with pullulan active packaging (a) mean values of malondialdehyde (MDA) contents at 0 day, 7th day and 14th day (b) overall MDA values of meat samples (c) impact of storage on MDA contents of meat samples and (d) impact of pullulan edible films on MDA contents of meat samples. [CTRL: without packaging; (b) PF-CTRL: treated with pullulan edible film; (c) PF-C-AgNPs: treated with pullulan edible film incorporated with curcumin arbitrated; (d) PF-P-AgNPs: treated with pullulan edible film incorporated with pullulan mediated silver nanoparticles; (e) PF-M-AgNPs: treated with pullulan edible film incorporated with mixed silver nanoparticles]

**Table 4.** Effect of pullulan active packaging on the oxidative stability of broiler breast meat (*Pectoralis major*).

	mg MDA, kg			ABTS%			DPPH%		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
CTRL	4.65 <sup>a</sup>	8.01 <sup>a</sup>	10.93 <sup>a</sup>	64.36 <sup>c</sup>	49.92 <sup>c</sup>	45.31 <sup>d</sup>	45.13 <sup>b</sup>	44.88 <sup>a</sup>	11.44 <sup>b</sup>
PF-CTRL	1.56 <sup>c</sup>	7.56 <sup>a</sup>	10.81 <sup>a</sup>	78.09 <sup>bb</sup>	58.73 <sup>bb</sup>	46.71 <sup>d</sup>	44.46 <sup>b</sup>	42.52 <sup>a</sup>	9.98 <sup>b</sup>
PF-C-AgNPs	1.32 <sup>d</sup>	3.04 <sup>c</sup>	5.53 <sup>d</sup>	91.87 <sup>a</sup>	77.32 <sup>a</sup>	73.91 <sup>a</sup>	60.080 <sup>a</sup>	44.50 <sup>a</sup>	18.76 <sup>a</sup>
PF-P-AgNPs	2.95 <sup>b</sup>	5.26 <sup>b</sup>	7.84 <sup>c</sup>	81.31 <sup>bb</sup>	61.99 <sup>b</sup>	60.71 <sup>b</sup>	63.80 <sup>a</sup>	35.86 <sup>b</sup>	15.49 <sup>a</sup>
PF-M-AgNPs	4.28 <sup>a</sup>	6.01 <sup>b</sup>	9.09 <sup>b</sup>	86.58 <sup>b</sup>	54.50 <sup>Bc</sup>	51.64 <sup>c</sup>	54.15 <sup>ab</sup>	36.07 <sup>b</sup>	15.46 <sup>a</sup>
SEM <sub>p</sub>	0.20	0.27	0.31	1.46	1.52	1.57	1.52		
p-Value	.000	.000	.000	.000	.000	.000	.000		

Means were compared at  $p < .05$ . The values with the same superscripts, within the same column, are not significantly different. CTRL: without packaging; PF-CTRL: treated with pullulan edible film; PF-C-AgNPs: treated with pullulan edible film incorporated with curcumin arbitrated AgNPs; PF-P-AgNPs: treated with pullulan edible films incorporated with pullulan mediated AgNPs; PF-M-AgNPs: treated with pullulan edible film incorporated with mixed silver nanoparticles. SEM<sub>p</sub>: Pooled standard error of means.



**Figure 8.** Free radical scavenging activity of broiler breast meat treated (*Pectoralis major*) treated with pullulan active packaging. [CTRL: without packaging; (b) PF-CTRL: treated with pullulan edible film; (c) PF-C-AgNPs: treated with pullulan edible film incorporated with curcumin arbitrated ; (d) PF-P-AgNPs: treated with pullulan edible film incorporated with pullulan mediated silver nanoparticles.; (e) PF-M-AgNPs:treated with pullulan edible film incorporated with mixed silver nanoparticles].

higher ( $p < .05$ ) MDA concentration was recorded in the broiler meat samples without packaging (CTRL) and with PF-M-AgNPs followed by PF-CTRL, PF-C-AgNPs and PF-P-AgNPs treatment groups at day 0 of the refrigerated storage. Significant differences ( $p < .05$ ) were noted among the controlled groups (CTRL, PF-CTRL) and treatment groups (PF-C-AgNPs, PF-P-AgNPs, PF-M-AgNPs) with respect to their MDA concentrations at 7th and 14th day of storage (Figure 7(a,d); Table 4).

### 2,2-Diphenyl-1-picryl hydrazyl (DPPH)

In this assay, 2,2-diphenyl-1-picryl hydrazyl or DPPH reagent is used to determine the free radical scavenging in its alcoholic reduction and  $H^+$  ions donation by any antioxidant (Sultana et al. 2017; Govindappa et al. 2018). In our study, significantly higher ( $p < .05$ ) DPPH radical scavenging activity was recorded in the meat samples treated with PF-C-AgNPs active packaging at day 0 ( $60.08 \pm 3.21\%$ ), day 7 ( $44.50 \pm 5.34\%$ ), and day 14 ( $18.76 \pm 2.84\%$ ) of the refrigerated storage followed by the meat samples treated with PF-P-AgNPs and PF-M-AgNPs active packaging (Figure 8; Table 4). No significant differences ( $p < .05$ ) were recorded in free radical scavenging activity between the PF-CTRL and CTRL treatment groups. DPPH radical scavenging activity was reduced with the advancement of the refrigerated storage ( $4 \pm 1^\circ C$ ) and was significantly higher ( $p < .05$ ) at day 0 ( $53.52 \pm 10.75\%$ ) and day 7th ( $40.77 \pm 5.62\%$ ) as compared day 14th ( $14.23 \pm 4.23\%$ ) of the storage (Table 4). The results of our study prove that DPPH radical scavenging activity of pullulan active meat packaging is a time dependant entity in the presence of green AgNPs.

### 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay

Another scientifically renowned approach for the detection of free radicals is ABTS assay and most of the food scientists rely on this assay along with DPPH assay (Ruiz-Cruz et al. 2019; Sultana et al. 2017). The detailed information, regarding the ABTS free radical scavenging activity percentages, is provided in Table 4.

PF-C-AgNPs active meat packaging generated statistically higher ( $p < .05$ ) ABTS radical scavenging activity at day 0 ( $91.87 \pm 7.09$ ), day 7th ( $77.32 \pm 3.52$ ) and day 14th ( $73.91 \pm 0.34$ ) of the refrigerated storage of broiler meat (Figure 8). Similarly, PF-P-AgNPs and PF-M-AgNPs active packaging exhibited more ABTS free radical scavenging capacity as compared to CTRL and PF-CTRL treatment groups (Figure 8; Table4). A significantly higher ( $p < .05$ ) ABTS free radical activity was recorded at day 0 ( $80.44 \pm 10.34$ ) as compared to the activity at day 7th ( $60.49 \pm 10.78$ ) and day14th ( $55.66 \pm 11.11$ ) of refrigerated storage of broiler meat. The overall trend of the free radical activity of all the treatment groups was parallel to that of DPPH assay as PF-C-AgNPs > PF-P-AgNPs > PF-M-AgNPs > PF-CTRL > CTRL (Figure 8).

## Discussion

### Meat colour

Many of the meat scientists agreed upon the fact that if the broiler meat is categorized as 'lighter', the pH and the cooking losses of that meat will be lower as compared to the 'darker ( $L^* + \text{mean} < 50$ )' broiler meat due to a correlation between these meat

qualities (Allen et al. 1998; Petracci et al. 2004). The colour of broiler meat is considered as one of the most significant quality parameters to determine the consumer's demand and acceptability (Garba et al. 2019). It can be correlated with the meat hardness or softness, perishability, microbial infestation, ageing and water holding capacity (Di Bernardini et al. 2011; Kozioł et al. 2015; Noor et al. 2018). The results of our study corroborate with the reports by Khare et al. (2017) for the increased 'darkness' of poultry meat during storage and Azlin-Hasim et al. (2016) who reported the effect of silver nanoparticles (Ag)/polyethylene nanocomposite films on the meat quality and colour characteristics during the refrigerated storage. The increase in the lightness reflects the maximum absorbance of curcumin arbitrated silver nanoparticles (C-AgNPs) into meat with better anti-oxidant capacity as compared to pullulan mediated silver nanoparticles (P-AgNPs) during the storage period (El-Refai et al. 2018). It is obvious that the green C-AgNPs save the denaturing and damage of the meat muscle proteins especially the 'myofibrillar' and 'sarcoplasmic' proteins resulting in lighter meat (Angsupanich and Ledward 1998; Ledward 1998; Azlin-Hasim et al. 2016). The red and yellow colour intensities of broiler meat are due to the maintenance of its 'pigment stability' as the transition of meat 'myoglobin' into 'ferrylmyoglobin' is inhibited by the anti-oxidant present in the treatment (Tesoriere et al. 2007; Zhang et al. 2016). The conversion of 'myoglobin' into 'ferrylmyoglobin' induces the lipid oxidation and the generation of free radicals resulting in a substantial damage to meat tactile characteristics with a reduction in the oxidative stability and shelf life of meat (Zhang et al. 2016).

### **pH of meat**

pH of broiler breast meat can be considered as a prominent marker to assess the quality, ageing and safety of poultry meat (Ruiz-Cruz et al. 2019). The increment in pH values indicates the propagation of 'proteolytic' bacterial strains during the storage period whereas the lactic acid producing bacterial strains may decrease the pH of breast meat (Ruiz-Cruz et al. 2019). The lowest pH of the meat treated with PF-C-AgNPs coincide with its 'lightness' of the colour as discussed earlier. It clearly reflects the continuance of 'oxidative stability' (with respect to lipids and proteins) of the broiler meat treated with PF-C-AgNPs active packaging. It seems that the limitation of the bacterial contamination along with the maintenance of meat

colour and pH is facilitated with the application of an active meat packaging (Abdulla et al. 2017; Cullere et al. 2018). Ruiz-Cruz et al. (2019) and Márquez-Ríos et al. (2011) demonstrated that the increased lactic acid concentrations in the broiler meat muscle, due to 'lactic acid producing bacteria', can decrease pH from 5.8 during anaerobic glycolysis. Similarly, higher nitrogen compound concentrations can increase pH of the broiler meat from 6.89 due to the deterioration of protein by certain 'proteolytic' bacterial strains (Cao et al. 2013; Ruiz-Cruz et al. 2019). The application of pullulan active packaging, incorporate with green silver nanoparticles (Ag) maintained their release from pullulan edible film to meat surface and reduced the propagation of glycolytic as well as proteolytic bacterial strains (Khalaf et al. 2013; Morsy et al. 2014; Panea et al. 2014; Cantalejo et al. 2016). The results of our study are in agreement with the reports presented by Carvalho et al. (2017) and Cantalejo et al. (2016) regarding the maintenance of pH of poultry meat in the range of 5.81 to 6.58 during storage period.

### **Water holding capacity**

The assessment of water retention of meat muscle fibres by 'expressible fluids' is one of the major quality indicators providing a synopsis of meat intracellular and extra cellular loose water vault (Yang et al. 2018). In our study, the broiler breast meat treated with PF-C-AgNPs reflected significantly ( $p < .05$ ) higher expressible fluids ( $59.17 \pm 11.22$ ) along with lowest drip losses ( $1.69 \pm 1.02$ ) as compared to other treatment groups i.e. CTRL, PF-P-AgNPs, and PF-M-AgNPs. It is established that the higher expressible fluid percentages reflect the better meat muscle integrity and water stability of meat indicating its improved quality (Ali et al. 2015; Kamruzzaman et al. 2016; Yang et al. 2018). Due to the microbial infestation of poultry meat surface, the proteolysis of myo-fibrillar proteins and collagens occurs resulting in the release of intercellular fluids during the ageing process (Pearce et al. 2011; Roslan et al. 2019). The lowest drip loss percentages reflects the lower microbial infestation and hence less protein denaturalisation that was noticed in PF-C-AgNPs treatment group. Moreover, hydrophilic active packaging of pullulan, incorporated with Ag nanoparticles, reduces the drip losses due to the electrostatic interaction between Ag nanoparticle and OH group of pullulan (Trinetta et al. 2011; Bahrami et al. 2019). This electrostatic interaction forms a thicker water and light barrier between the meat surface and the environment limiting the chances of meat spoilage during

storage (Khalaf et al. 2013; Bahrami et al. 2019). The results of our study are in accordance with the reports by Roslan et al. (2019) and Khare et al. (2017) with respect to the increased drip losses (DL) of meat during ageing and refrigerated storage.

Water holding capacity (WHC) of meat is considered as one of the necessary attributes for its quality and acceptability by the consumers (Carvalho et al. 2017; Ruiz-Cruz et al. 2019). The meat pH, texture, and muscle protein stability (myofibrillar, myosin) are directly affected by the water retention (WHC) in the muscle filament and inter-cellular spaces (Young and Buhr 2000; Young et al. 2004; Hashemi et al. 2014; Ruiz-Cruz et al. 2019). The meat samples treated with PF-C-AgNPs ( $86.49 \pm 3.34$ ) exhibited a significantly higher WHC ( $p < .05$ ) than the CTRL ( $79.92 \pm 6.1$ ). It was due to the retention of muscle cell integrity in broiler breast meat resulting in the lower oxidation of protein tissues in the presence of a good anti-oxidant substance (Hashemi et al. 2014). The incorporation of curcumin arbitrated green Ag nanoparticles into pullulan edible films maintained the 'meat anti-oxidant defence' mechanism resulting in a higher WHC percentage. The better WHC may be due to the lower protein de-naturalization by microbes or any other environmental factor (Young et al. 2004; Ruiz-Cruz et al. 2019). It can increase the meat shelf life as well as the customer acceptability (Young et al. 2004; Ruiz-Cruz et al. 2019). These results are in agreement with those reported by Carvalho et al. (2017) who reported WHC percentages of poultry meat in the range of 79 to 86% by compression method.

### **Meat texture**

The texture analysis reflected that the softness or gumminess of broiler meat decreased with the advancement of the storage duration (from day 0 to day 14). The broiler meat treated with pullulan active packaging reflected a significant ( $p < .05$ ) increase in the softness of the texture (PF-C-AgNPs > PF-P-AgNPs > PF-M-AgNPs) and significant ( $p < .05$ ) reduction in the shear energy or elasticity (PF-C-AgNPs < PF-P-AgNPs < PF-M-AgNPs) as compared to the control treatment groups (CTRL, PF-CTRL). Roslan et al. (2019) and Küçüközet and Uslu (2018) reported that the softness of poultry meat (without any active packaging) or its gumminess is directly influenced by the increased storage duration which coincided with our findings. Similarly, our results are in agreement with the reports by Bowker and Zhuang (2019), Roslan et al. (2019) and Küçüközet and Uslu (2018) with

respect to the amplified shear energy ( $N \times mm$ ) or elasticity of poultry meat with increased storage duration. When a force is applied on the meat muscle tissues, a parallel energy is also generated by the meat reflecting the chewiness or elasticity of muscle fibres (Bowker and Zhuang 2019; Development 2019). The chewiness is declined due to increased muscle rigidity and harness of the surface of the meat resulting in the tougher and elastic meat (Bowker and Zhuang 2019). Our study demonstrated that the textural characteristics of broiler meat, treated with PF-C-AgNPs, are consequently modified into softer, gummier, chewable or less elastic during the refrigerated storage. Furthermore, the pullulan active packaging maintained the compositional integrity of the broiler meat muscle fibres and connective tissues with a limited or nominal chemical change (Soglia et al. 2016). It seems that the application of PF-C-AgNPs, restrained the 'fibrosis' or protein denaturalisation after cooking which limited the muscle fibre shrinkage and accumulation of 'collagen' in muscle tissues. The accumulated 'collagen' (by fibrosis) can modify the tactile features of the meat but also indicates the compositional deviations and denaturalisation of meat muscles (Huff-Loneragan and Lonergan 2005; Soglia et al. 2016).

### **Oxidative stability of meat**

Lipid per-oxidation of broiler meat can be evaluated by the formation of thiobarbituric acid reactive substances (TBARS) during the refrigerated storage providing a clear picture about the oxidative stability in meat (Sasaki et al. 2008; Vital et al. 2016, 2018). The lowest MDA concentrations in poultry meat, treated with PF-C-AgNPs active packaging, reflects that the thin layer of curcumin around C-AgNPs enhanced the anti-oxidant ability of pullulan edible film resulting in an improved oxidative stability. The concentration of 'melanodialdehydes' (mg MDA/kg of meat) detected in our study is in agreement with the findings of Souza and Fernando (2016), Vital et al. (2018), and Vital et al. (2016) who suggested that the edible coatings, incorporated with natural anti-oxidants, could maintain the oxidative stability in poultry meat as compared to the controlled samples (without coatings). The malondialdehyde (MDA) concentrations of broiler meat, in our study, are far below than the reports of Rababah et al. (2006) who evaluated the effect of plant extract on lipid oxidation (17.3–80.1 mg MDA/kg) of raw poultry meat for 12 days of refrigerated storage. Furthermore, the results of our study (1.32–10.93 mg MDA/kg) are also less than the findings of Flavia et al. (2014) who



found that the refrigerated storage time vigorously affect the lipid oxidation in poultry meat (2.16 to 14.60 mg MDA/kg) after 24 h of storage. The pullulan active packaging, containing glycerol as plasticiser, has a good synergism with green AgNPs to boost the oxygen and water barrier qualities (Khalaf et al. 2013; Trinetta and Cutter 2016). Due to the oxygen barrier property, the active packaging, incorporated with green anti-oxidant substances, exhibits a stronger anti-oxidant capability during refrigerated storage (Liu et al. 2015; Raeisi et al. 2015). The results of our study suggest that the quality and the shelf life of broiler meat, treated with PF-C-AgNPs, PF-P-AgNPs and PF-M-AgNPs active packaging, can be maintained thoroughly during the refrigerated storage (14 days) as compared to the meat without packaging (Vital et al. 2016). In addition, the determination of free radicals by DPPH and ABTS scavenging activities is another approach to evaluate the oxidative rancidity or lipid oxidation of broiler meat (Sultana et al. 2017).

The green and environment friendly C-AgNPs, surrounded by curcumin, can bind the maximum number of the free radical ions due to their donation of electrons and provide an excellent anti-oxidant capacity to PF-C-AgNPs and PF-P-AgNPs (Falowo et al. 2014; Vital et al. 2016; El-Refai et al. 2018; Khan, Kumari, et al. 2019; Khan et al. 2020). The results of the current study are in the agreement with Ruiz-Cruz et al. (2019), Sultana et al. (2017) and Zhang et al. (2016) for a reduced free radical scavenging activity in the controlled poultry meat samples along with the effect of storage duration on DPPH radical scavenging percentages. Moreover, ABTS scavenging activity reflects that the green synthesised C-AgNPs and P-AgNPs can boost the anti-oxidant potential of PF-C-AgNPs and PF-P-AgNPs with better oxidative stability. It seems that PF-C-AgNPs and PF-P-AgNPs active packaging are capable for the reduction of broiler meat oxidative rancidity during a refrigerated storage period of 14 days. Ruiz-Cruz et al. (2019) and Sultana et al. (2017) reported the similar results of ABTS free radical scavenging activity with lowest values for the controlled treatment groups.

## Conclusions

Based on the findings of our study, it can be concluded that the oxidative stability of meat treated with PF-C-AgNPs active packaging is significantly ( $p = .000$ ) better with lowest concentrations of 'malondialdehydes' (mg MDA/kg of meat) at day 0 ( $1.32 \pm 0.29$ ), day 7th ( $3.04 \pm 0.61$ ) and day 14th

( $5.53 \pm 0.72$ ). Similarly, significantly higher ( $p < .05$ ) pH, drip losses, cooking losses, and shear energy ( $N \times mm$ ) of the control treatment groups (CTRL, PF-CTRL) reveal that the green synthesised curcumin arbitrated silver nanoparticles (C-AgNPs) and pullulan mediated silver nanoparticle (P-AgNPs), incorporation into pullulan edible films, potentially maintained the broiler meat quality during refrigerated storage ( $4 \pm 1$  °C). Moreover, 'pullulan active meat packaging', incorporated with C-AgNPs and P-AgNPs, can be considered as a green and bio-degradable substitution of the synthetic and hazardous anti-oxidant packaging for prolonging broiler meat quality and shelf life.

## Scope, limitations, and recommendations

Pullulan active meat packaging, incorporated with green C-AgNPs and P-AgNPs, will be safer and simpler antioxidant substitute against the hazardous and synthetic antioxidants. Implementation of real time commercial equipments, huge capital investment, slow processing, and time consumption at laboratory level are the major limitations of the current study. Life cycle assessment (LCA) and antimicrobial activity of pullulan active packaging incorporated with C-AgNPs and P-AgNPs are the recommendations to evaluate the impact of nanoparticles on human beings, ecosystem, and various microbial species.

## Ethical approval

This project was conducted following the animal ethics and protocols approved by 'Institutional Animal Care and Use Committee' of the Universiti Putra Malaysia, Malaysia (UP M/ IACUC/AUP-R088/2018).

## Disclosure statement

No potential competing interest was reported by the author(s).

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## Data availability statement

The data used to support the findings of this study are included within the article.

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