Effect of pullulan active packaging, incorporated with silver nanoparticles, on cholesterol oxidation product concentrations in boiler meat during storage

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Abstract

Cholesterol oxidation products (COPs) in meat are thrombogenic, mutagenic, atherogenic, carcinogenic, angiotoxic, and cytotoxic, leading to serious health issues. The formation of COPs in meat is induced by oxidative rancidity due to poor meat processing and packaging techniques. Pullulan active packaging, incorporated with silver nanoparticles (AgNPs), is considered strong and biodegradable with characteristics, such as enhanced light, gas, and moisture barrier, that protect the oxidative rancidity of broiler meat. The current study was performed to determine the impact of pullulan active packaging (T1, T2, T3, and T4) on the formation of COPs in broiler meat during refrigerated storage (7 and 14 days). Pullulan active packaging significantly affected ($P < 0.05$) the concentrations of A-cholestane (0.63±0.10 ppm) and B-epoxy (0.59±0.33 ppm) COPs whereas A-cholestane (0.634±0.08 ppm) was affected significantly ($P < 0.05$) by the duration of refrigerated storage (0, 7, and 14 days). Broiler meat treated with pullulan active packaging presented a limited level of oxidative rancidity with minimum concentrations of COPs. The findings revealed that broiler meat treated with pullulan active packaging, incorporated with AgNPs, is safer than raw meat for shorter (7 days) and longer (14 days) storage periods at 4±1°C.

Keywords: cholesterol; active meat packaging; cardiovascular diseases; broiler meat; pullulan

Introduction

Cholesterol is considered an important compound of animal origin (meat), which constitutes the membranes of cells (Khan *et al.*, 2015; Li *et al.*, 2019). The total amount of cholesterol in meat is mainly governed by the type of meat (species), its chemical composition (cuts), and its total fat content (Ahmad *et al.*, 2018; Serra *et al.*, 2014). Many scientists reported a strong relationship between food safety, human health, and intake of fat contents of meat, that is, polyunsaturated fatty acids (PUFAs) (Barbaro, 2022; Khan *et al.*, 2015; Serra *et al.*, 2014). Excessive concentration of PUFAs in meat is prone to 'photo-oxidation, enzymatic oxidation, or autoxidation oxidation', leading to the formation of cholesterol oxidation products (COPs) during meat processing and storage (Nadia, 2019). Double bonding present at Δ-5 in the chemical configuration of PUFA intensifies oxidation process, resulting in COPs (Nadia, 2019).

The oxidation process in all 70 known types of cholesterols is initiated by the preoccupation of hydrogen molecules at the C-7 point of their chemical structure with the subsequent addition of oxygen molecules (Xu and Porter, 2015). The formation of 7A-hydroperoxycholesterol (7A-OOH) and 7B-hydroperoxycholesterol (7B-OOH)

is caused by oxidation process, leading to their breakdown into 7A-hydroperoxycholesterol (7A-OH) and 7B-hydroxycholesterol (7B-OH) (Zerbinati and Iuliano, 2017). During processing, storage, or heating, both of these isomeric hydroxycholesterols formulate 7-ketocholesterol (7-keto) and 25-OH (Rather *et al.*, 2021).

According to different studies, COPs are considered thrombogenic, mutagenic, atherogenic, carcinogenic, angiotoxic, and cytotoxic for humans, and they cause serious health issues (Khan *et al.*, 2015; Serra *et al.*, 2014). Owing to the formation of potentially reactive aldehydes in tissues and cells, COPs are the major cause of blood artery choking by fatty plaques, earlier aging, prominent arthritis, and cardiovascular, Parkinson's and Alzheimer's diseases in humans (Choe *et al.*, 2018; Milićević *et al.*, 2014).

As a nutritionally important part of human diet, broiler meat contains relatively higher concentrations of PUFAs and needs more attention to limit the chances of cholesterol oxidation (Choe *et al.*, 2018). Similarly, the consumption of fresh broiler meat is highly appreciated by many researchers, compared to processed or stored meat to prevent health and nutritional risks (Choe *et al.*, 2018). To prevent the formation of COPs in broiler meat, many techniques have been reported by scientists, including nutritional manipulation and supplementation, meat processing, refrigerated storage (for 14 days), vacuum packaging, modified atmosphere packaging, and active packaging (Khan *et al.*, 2015, 2020; Morsy *et al.*, 2014; Mousavi Khaneghah *et al.*, 2018).

To prevent COP generation in meat and meat products, the use of antioxidants, three essential oils (olive oil, linseed oil, and rosemary oil), plant extracts (grape extract, avocado extract, and rosemary extract), and packaging technologies (active and intelligent) have been reported in the last decade (Khan *et al.*, 2022; Macho-González *et al.*, 2021; Manzoor *et al.*, 2022). It is suggested that meat and meat products stored in biodegradable active packaging symbolizes more oxidative stability and low microbial loads (Khan *et al.*, 2022; Noor *et al.*, 2018).

Among the most utilized nanoparticles in active packaging to enhance the shelf life of food are silver nanoparticles (AgNPs) (Mohammed *et al.*, 2023). According to the research conducted by Echegoyén and Nerín (2013), transfer of silver (Ag) from food packaging to food depends on food type and warming. However, the authors concluded that migration of Ag is below the maximum migration limits recognized by European Union (EU) legislation. This finding was supported by Gallocchio *et al.* (2016), who did not observe Ag content higher than the permitted limit by the EU in Ag packages of chicken breast.

Pullulan active packaging, incorporated with any active substance, such as AgNPs, titanium oxide nanoparticles (TiO NPs), gold nanoparticles (AuNPs), or plant extracts, reflects strong oxygen barrier qualities (Khan *et al.*, 2019, 2020; Trinetta and Cutter, 2016). Owing to the strong oxygen barrier capacity, well-built intermolecular hydrogen bonding and a slower release of active pullulan packaging compounds reduce the chances of cholesterol oxidation in preserved meat (Khan *et al.*, 2020; Rai *et al.*, 2021). Development of innovative food packaging, such as pullulan active packaging, is aimed at meeting the societal demand of safe and high-quality food products. This is consistent with the resolution of the food industry to achieve a unique quality of food packaging to improve global food security.

The current study was conducted to investigate the concentrations of COPs in broiler meat treated with different types of pullulan active packaging treatments, stored at 4±1°C for 0, 7, and 14 days. These active packaging included pullulan active packaging incorporated with curcumin-mediated AgNPs (PF-C-AgNPs), pullulan active packaging incorporated with pullulan-mediated AgNPs (PF-P-AgNPs), and pullulan active packaging incorporated with mixed AgNPs (PF-M-AgNPs).

Materials and Methods

Broiler meat production

All protocols and ethics of this study were approved by the Institutional Animal Care and Use Committee of the University Putra Malaysia, Malaysia (UPM/IACUC/AUP-R088/2018).

Preparation of silver nanoparticles and pullulan active packaging

Two types of AgNPs were formulated as pullulan-mediated AgNPs (P-AgNPs) with ultraviolet (UV) irradiation, as reported by Khan *et al.* (2019), while curcumin-mediated AgNPs (C-AgNPs) were synthesized by chemical reduction according to the procedures reported by Khan *et al.* (2019). An equal quantity of colloidal solution of P-AgNPs and C-AgNPs was utilized to obtain mixed AgNPs (M-AgNPs) for active packaging. Similarly, pullulan active packaging was synthesized by incorporating 2% (v/v) P-AgNPs and C-AgNPs into pullulan edible film according to the procedure reported by Khan *et al.* (2022).

Treatment groups

Two control groups and three treatment groups were generated in this study, that is, control negative (C0;

without active packaging), PF-CTRL (T1: meat treated with pullulan active packaging without AgNPs), PF-C-AgNPs (T2; meat treated with pullulan active packaging incorporated with curcumin AgNPs), PF-P-AgNPs (T3; meat treated with pullulan active packaging incorporated with pullulan AgNPs), and PF-M-AgNPs (T4; meat treated with pullulan active packaging incorporated with mixed AgNPs). In all, 30 broiler birds were allocated to each treatment group. The obtained broiler breast meat (pectoralis major) samples were cleaned of all debris and fats, and stored at 4±1°C for 0, 7 and 14 days (Khan *et al.* 2015, 2022; Liu *et al.* 2019). For each replicate within the treatment group, 10 random meat samples (n = 10) were utilized. In active packaging, 2 g of chicken meat was wrapped, while the negative control was wrapped in aluminium foil and vacuum-packed by using chamber vacuum sealer VS168 (QuiWARE, KL, Malaysia; Khan *et al.*, 2022). The details of broiler meat samples (Pectoralis major) treated with pullulan active packaging for different storage periods are provided in Table 1.

Saponification of COPs

For saponification, the samples were placed in test tubes with caps. In all, 10 mL potassium hydroxide (KOH [1 M], 56.11 g + 95% ethanol) was added to the sample at room temperature (RT) for 22 h in the dark (Rahim *et al.*, 2012). For extraction, 5 mL of distilled water and 10 mL of hexane (>95%; Sigma-Aldrich, St. Louis, MO, USA) were added to the samples. The mixture was shaken using a vortex. The hexane fraction located in the upper layer was separated and transferred to another test tube. The samples were placed in a water bath (45°C) and allowed to dry up to a specific level. Extraction with 10 mL of hexane was repeated for two times until equilibrium level was reached (Choe *et al.*, 2018). The sample was subsequently dried under nitrogen flow. After drying, 1 mL of hexane was added to the test tube and shaken. Then the samples were transferred to an amber bottle, sealed with parafilm, and placed at -18°C (Choe *et al.*, 2018).

Table 1. Meat samples (Pectoralis major) treated with pullulan active packaging at different storage periods.

C0	Control sample for 0 day
C7	Control sample for 7 days
$T_1$7	Treatment 1 for 7 days
$T_2$7	Treatment 2 for 7 days
$T_3$7	Treatment 3 for 7 days
$T_4$7	Treatment 4 for 7 days
C14	Control sample for 14 days
$T_1$14	Treatment 1 for 14 days
$T_2$14	Treatment 2 for 14 days
$T_3$14	Treatment 3 for 14 days
$T_4$14	Treatment 4 for 14 days

Derivatization of COPs

The samples were filtered using the nylon syringe filter unit Millex (0.45 µm × 13 mm). The reference standard (1 mg) was dissolved in 1-mL hexane and ultrasonicated. Then the samples were filtered using a Millex nylon syringe filter unit (0.45 µm × 13 mm). The samples and reference standard were stored at -20°C in amber bottles (with blue caps). Discovery of COP derivatives was completed by gas chromatography mass spectrometry–triple quadropole (GCMS-QQQ) quantification within 1 week (Choe *et al.*, 2018; Synowiec *et al.*, 2014).

Data analysis

One-way analysis of variance (ANOVA) was performed for data analysis by using SAS software (9.40, SAS Institute, NC, USA). Significant differences between mean values were obtained by Duncan's test, and the results were expressed as mean value and standard error of mean (SEM) (Choe *et al.*, 2018). The coefficient of variation (*R*) was observed to check correlation between mean values by using the SAS software (9.40, SAS Institute) (Choe *et al.*, 2018; Khan *et al.*, 2022).

Results and Discussion

The impact of pullulan active packaging treatment and duration of refrigerated storage (4±1°C) on the quantity of COPs is provided in Tables 2 and 3. In all, 110 observations were created as per treatment (control, T1, T2, T3, and T4) with three storage durations (0, 7, and 14 days). The concentrations of B-epoxy, A-cholestane, and 7-keto COPs were significantly ($P < 0.05$) affected by the application of active packaging and days of refrigerated storage. Moreover, a strong coefficient of variation (*R*) was observed between different concentrations of COPs (A-epoxy, B-epoxy, A-cholestane, 7-keto, and 25-OH) and pullulan active packaging treatments with durations of storage (Tables 2 and 3).

In the present study, the level of additional functional groups of COPs in the sample was influenced by the storage duration (at 4±1°C). In relation to storage time and treatment, the concentration (ppm) of A-cholestane was significantly different ($P < 0.05$), compared to that of cholesterol, B-epoxy, A-epoxy, 7-keto, and 25-OH.

Broiler meat samples treated with pullulan active packaging incorporated with silver NPs (PF-P-AgNPs,

Table 2. Cholesterol oxidation products (COPs) in broiler meat treated with pullulan active meat packaging treatment incorporated with silver nanoparticles (AgNPs).

Cholesterol oxidation products (COPs)	Pullulan active meat packaging incorporated with AgNPs					COP's mean values	ANOVA		Coefficient of variation (*R*)
	Control (no packaging)	T_1 (PF-CTRL)	T_2 (PF-C-AgNPs)	T_3 (PF-P-AgNPs)	T_4 (PF-M-AgNPs)		F_{value}	*P*	
A-epoxy (ppm)	14.23 ± 4.66^{A}	23.0 ± 5. 42^{A}	24.65 ± 5.40^{A}	18.13 ± 5.77^{A}	15.164 ± 5.26^{A}	18.70 ± 5.0^{NS}	0.98	0.4651	119.03
B-epoxy (ppm)	0.303 ± 0.319^{B}	0.31 ± 0.37^{B}	0.31 ± 0.37^{B}	1.38 ± 0.38^{A}	0.785 ± 0.35^{A,B}	0.59 ± 0.33^{S}	2.04	0.0394	247.90
A-cholestane (ppm)	0.3887 ± 0.139^{B}	0.290 ± 0.17^{B}	0.2820 ± 0.18^{B}	1.3100 ± 0.17^{A}	1.0210 ± 0.18^{A}	0.63 ± 0.10^{S}	15.62	<0.0001	88.48
Cholesterol (ppm)	24.74 ± 13.80^{A}	38.49 ± 14.9^{A}	35.74 ± 16.10^{A}	21.59 ± 17.50^{A}	67.73 ± 16.76^{A}	36.6 ± 13.4^{NS}	1.04	0.4234	159.30
7-keto (ppm)	1.73 ± 0.24^{A}	2.072 ± 0.29^{A}	1.9260 ± 0.39^{A}	1.6285 ± 0.395^{A}	2.2825 ± 0.40^{A}	1.93 ± 0.23^{NS}	1.98	0.0438	64.81
25-OH (ppm)	4.23 ± 3.76^{A}	3.34 ± 7.03^{A}	7.80 ± 3.51^{A}	0.69 ± 5.74^{A}	3.64 ± 3.76^{A}	4.66 ± 4.28^{NS}	0.52	0.8281	219.45

T_1 = PF-CTRL-pullulan packaging as a positive control); T_2 = PF-C-AgNPs (pullulan active packaging with curcumin silver nanoparticles); T_3 = PF-P-AgNPs (pullulan active packaging with pullulan-mediated silver nanoparticles); T_4 = PF-M-AgNPs (pullulan active packaging with mixed [curcumin and silver] nanoparticles).
Mean values ± standard error of mean (SEM) were compared at $P < 0.05$.
Mean values with same superscript letter are not significantly different.
NS = nonsignificant difference, S = significant difference.

PF-C-AgNPs, and PF-M-AgNPs) reflected comparatively lower concentrations of COPs than the meat samples treated with pullulan packaging (PF-CTRL; Figure 1). A higher concentration of A-epoxy (24.65±5.40 ppm) and 25-OH (7.80±3.51 ppm) was observed in broiler meat samples treated with T2 (PF-C-AgNPs) whereas the meat samples treated with T3 (PF-P-AgNPs) provided higher concentrations of B-epoxy (1.38±0.38 ppm) and A-cholestane (1.3100±0.17 ppm; Figure 1). The C0 (control) and T1 (PF-CTRL) treatment groups showed similar levels (ppm) of A-epoxy, B-epoxy, A-cholestane, cholesterol, 7-keto, and 25-OH COPs during storage periods (Figure 1 and Table 2). On day 14 of storage, the concentration of A-cholestane (0.634±0.08 ppm) was significantly affected ($P \leq 0.0001$) by storage period in all treatment groups (Table 3). In the present experiment, no significant effect of storage period ($P < 0.05$) was observed on the concentrations (ppm) of A-epoxy, B-epoxy, cholesterol, 7-keto, and 25-OH COPs, except that their concentrations were maximum on day 7 of refrigerated storage (Figure 2).

Higher values of coefficient of variation ($R > 50$) reflected an increased impact of pullulan active packaging treatment and duration of storage on PUFA oxidation in broiler meat (Tables 2 and 3).

In the present study, impact of different types of pullulan active packaging—pullulan active packaging incorporated with silver NPs (PF-P-AgNPs, PF-C-AgNPs, and PF-M-AgNPs)—was evaluated on raw broiler meat to address the potential threats of COPs to human health. The dual effects of storage period and treatment on COP generation in broiler meat were determined in our study. A positive association was observed between COP generation, length of storage, and broiler meat treatments (T1, T2, T3, and T4). The oxidation of PUFA in broiler meat was facilitated by storage period (7 and 14 days), enhancing the concentrations of COP, 7-keto, B-epoxy, and A-epoxy COPs (Choe *et al.*, 2018; Manzoor *et al.*, 2022). The oxidation of PUFA can be minimized by incorporating active ingredients (curcumin, linseed oil, fish oil, etc.) into meat products during refrigerated storage cholesterol (36.60±11.13), 7-keto (1.927±0.18), B-epoxy (0.593±0.21), A-epoxy (18.70±3.18), and 25-OH (4.657±2.50) in broiler meat stored for 14 days, compared to control meat samples on day 0 (Table 3). The oxidation of PUFA into COPs, especially A-cholestane, was significantly facilitated ($P \leq 0.0001$) by storage period. Our results showed agreement with the studies conducted by Choe *et al.* (2018), Ming-min and Ismail-Fitry (2023), and Yao *et al.* (2018) regarding the significant increase in COPs, which occurred with increase in the period of meat storage. This finding was consistent with the results demonstrated by Hashari *et al.* (2020), who reported that the degree of COP generation is affected by type of food

Table 3. Cholesterol oxidation products (COPs) in broiler meat treated with pullulan active packaging (ppm) at different storage durations (4±1°C).

Cholesterol oxidation products (COPs)	Duration of poultry meat storage			COP's mean values	ANOVA		Coefficient of variation (R)
	Day 0	Day 7th	Day 14th		F_{value}	*P*	
A-epoxy (ppm)	11.30 ± 7.45^{A}	19.20 ± 3.95^{A}	19.72 ± 3.19^{A}	18.70 ± 3.18^{NS}	0.55	0.5769	119.5236
B-epoxy (ppm)	0.30 ± 0.51^{A}	1.06 ± 0.26^{A}	0.31 ± 0.22^{A}	0.593 ± 0.21^{NS}	2.67	0.0751	257.0312
A-cholestane (ppm)	0.30 ± 0.25^{B}	0.31 ± 0.11^{B}	1.03 ± 0.11^{A}	0.634 ± 0.08^{S}	11.45	<0.0001	124.0265
Cholesterol (ppm)	18.24 ± 23.84^{A}	47.27 ± 10.32^{A}	29.37 ± 10.32^{A}	36.60 ± 11.13^{NS}	1.08	0.3469	159.5552
7-keto (ppm)	1.60 ± 0.41^{A}	2.11 ± 0.18^{A}	1.81 ± 0.18^{A}	1.927 ± 0.18^{NS}	1.00	0.3708	67.65666
25-OH (ppm)	3.28 ± 5.50^{A}	6.16 ± 2.18^{A}	0.60 ± 3.90^{A}	4.657 ± 2.50^{NS}	0.81	0.4555	204.6196

Mean values ± standard error of means (SEM) were compared at $P < 0.05$.
Means with the same superscript letter are not significantly different.
NS = nonsignificant difference, S = significant difference.

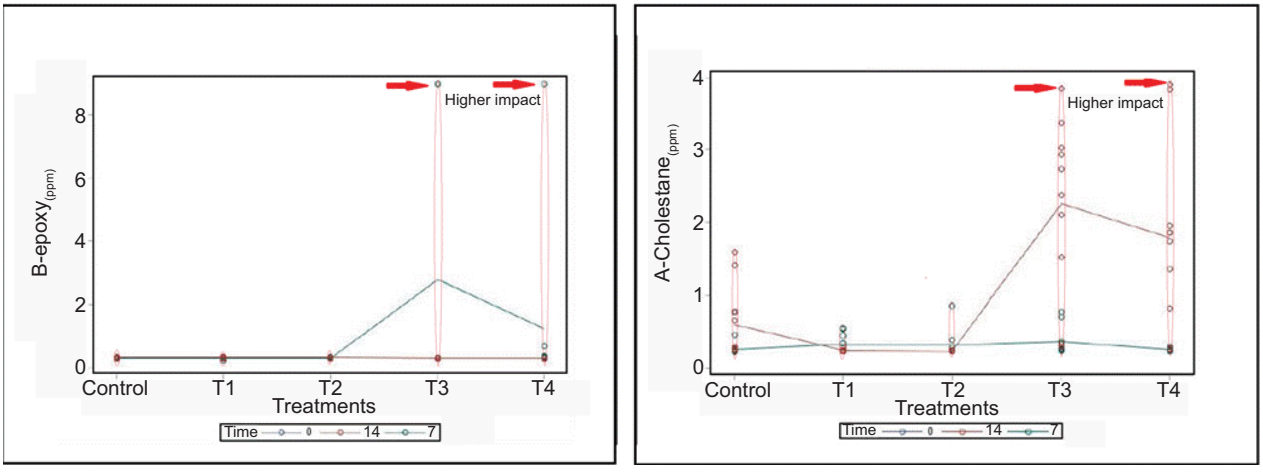

Figure 1. Interaction plots of cholesterol oxidation products (COPs) and pullulan active meat packaging treatments.

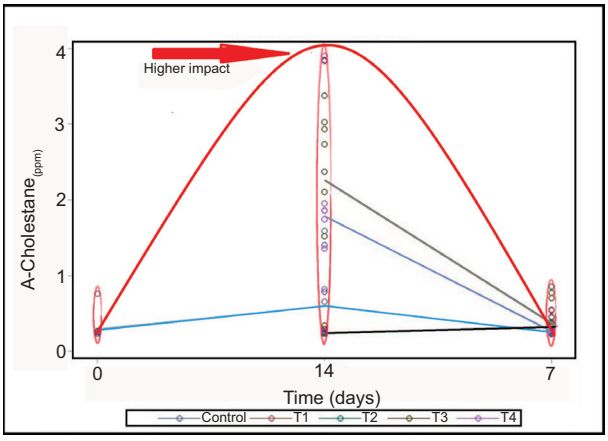

Figure 2. Interaction plots of cholesterol oxidation products (COPs) and storage periods.

packaging, storage conditions, cooking time, and cooking method.

The absorption of COPs in the digestive tracts of humans and animals is mediated by different mechanisms and is dependent on nature of the compound. The absorption rate of COP is better in animals because it is more polar than cholesterol (Derewiaka, 2022). Although the total amount of PUFA in broiler meat is approximately 1%, considerable care is necessary for the increased level of COPs because of oxidation (Ali *et al.*, 2015; Min *et al.*, 2015). If untreated broiler meat stored for a longer period is utilized, the absorption of unnecessary COPs is facilitated by intestinal lipophilic membranes, compared to cholesterol molecules (Ali *et al.*, 2015; Synowiec *et al.*, 2014). As a fraction of chylomicron structures,

COPs enter the bloodstream, causing potential hazards, including apoptosis of murine thymocytes, monocytes, and smooth muscle cells and necrogenic mechanisms in other vascular cells (Ali *et al.*, 2015).

To overcome the oxidative rancidity of PUFA present in broiler meat, meat processing and packaging is reported as a major tool for the safety of meat (Bennato, 2020; Choe *et al.*, 2018). The biological impact of pullulan active packaging on human health is quite minimal and can diminish the hazardous effects of COPs in humans (Khan *et al.*, 2020, 2022). The current study revealed that pullulan active packaging, incorporated with AgNPs (T1, T2, T3, and T4), restricted the concentration (ppm) of COPs because of oxidation of PUFA present in broiler meat during storage.

It is proved that photo-oxidation and auto-oxidation of broiler meat is prevented by pullulan active packaging (incorporated with any active substance) because of its better oxygen, light, and water barrier capacities (Khan *et al.*, 2019; Morsy *et al.*, 2014). For this reason, many food scientists suggest the utilization of pullulan active packaging, in addition to vacuum, for long- and short-term storage of broiler meat (Khan *et al.*, 2020, 2022).

Meat packaging (either active or vacuum) can minimize the formation of COPs, especially 7-keto, a-cholestane, B-epoxy, and A-epoxy (Serra *et al.*, 2014). A total of 23–37% of COPs comprise 7-keto and A-cholestane generated by the oxidation of cholesterols present in meat (Min *et al.*, 2015). A similar pattern was observed in the present study. Pullulan active packaging with treatments T1 (0.290±0.17; 0.31±0.37) and T2 (0.2820±0.18; 0.31±0.37) significantly ($P < 0.05$) maintained the concentrations (ppm) of A-cholestane ($P \leq 0.0001$) and B-epoxy ($P = 0.0394$) COPs. The concentrations of both COPs was similar to that of the control sample (C0) with no storage compared to treatments T3 (1.3100±0.17; 1.38±0.38) and T4 (1.0210±0.18; 0.785±0.35). No significant differences ($P < 0.05$) were observed for A-epoxy, cholesterol, 7-keto, and 25-OH COPs regarding meat treatment with pullulan active packaging. These results demonstrated agreement with the findings of the study conducted by Choe *et al.* (2018).

According to Choe *et al.* (2018), concentrations of COPs present in raw poultry meat, especially A-cholestane and cholesterol, are affected by meat treatment and/or storage period. Therefore, no significant differences ($P < 0.05$) were observed in the concentrations of A-epoxy, 7-keto, and 25-OH COPs after 6 days of storage. In our study, a similar pattern was observed for A-cholestane after the 7th day of refrigerated storage ($P < 0.0001$), as reported by Choe *et al.* (2018), but cholesterol followed the opposite pattern ($P = 0.4234$) (Choe *et al.*, 2018). The most hazardous 25-OH COP, presented in broiler meat, was not affected by the application of T1, T2, T3, and T4 active packaging, which contradicts the study conducted by Choe *et al.* (2018). The overall concentrations of 7-keto (1.6285–2.2825 ppm), A-epoxy (14.23–24.65 ppm), and B-epoxy (0.303–1.38 ppm) in raw and treated meat were within the normal range, similar to the studies conducted by Winiarska-Mieczan *et al.* (2016), and Yao *et al.* (2018).

The minimal conversion of PUFA into COPs during refrigerated storage is facilitated by temperature, broiler meat treatment, meat packaging, cholesterol–COP ratio in broiler meat, and vacuum packaging (Ramiah *et al.*, 2014; Yao *et al.*, 2018). The conversion of PUFA into COP is mainly induced by oxidation at sterol rings, particularly at the most sensitive sites (4, 5, 6, and 7), forming COPs (Nadia, 2019; Yao *et al.*, 2018). The methylene group (C-7 allylic site) of cholesterol is considered the initiation spot for cholesterol–COP conversion, resulting in the formation of isomers of COPs as '7-hydroperoxycholesterol' (Nadia, 2019). 7β-hydroxycholesterol (7β-OHCh) and 7α-hydroxycholesterol (7α-OHCh) COPs are subsequently formed by consuming more oxygen from the environment (Nadia, 2019; Ramiah *et al.*, 2014). In the present study, vacuum packaging, meat treatments (T1, T2, T3, and T4), and refrigerated storage of the treated meat prevented the conversion of PUFA to COPs because of limited oxygen supply, which was consistent with previous results (Mir *et al.*, 2017; Ramiah *et al.*, 2014; Yao *et al.*, 2018).

The oxidation of PUFA is amplified by the length of storage period, and pullulan active packaging has a tendency to minimize the concentrations of COPs (A-cholestane, cholesterol, 7-keto, A-epoxy, 25-OH, and B-epoxy) during refrigerated storage (Lee *et al.*, 2011; Mir *et al.*, 2017; Winiarska-Mieczan *et al.*, 2016). Although the obtained values did not reach the threshold, a comprehensive assessment was presented by our study for the healthy preservation of broiler meat.

Conclusions

The results of our study reflected that the oxidation of PUFAs into COPs in broiler meat is a time-dependent process. The conversion of PUFAs into COPs is clogged by effective meat treatments with certain degradable and green products, such as biodegradable active packaging. However, pullulan active packaging, incorporated with green synthesized AgNPs, minimize the oxidation process by efficient light, gas, and water barrier capacity. For this reason, COP generation is minimized during storage. The findings indicated that broiler meat treated with pullulan active packaging is safer for human

consumption than the stored meat. Hence, in addition to reducing the likelihood of meat perishability, pullulan active meat packaging is safe, effective, and reduces oxidative rancidity and COP production. Comprehensive studies are needed to elucidate the mechanism of pullulan active packaging incorporated with green synthesized AgNPs on COPs in different meat types under different conditions.

Author Contributions

Conceptualization: Suriya Kumari Ramiah; *methodology*: Suriya Kumari Ramiah, Muhammad Jamshed Khan, and Yashini Subramaniam; *validation*: Muhammad Jamshed Khan and Suriya Kumari Ramiah; *formal analysis*: Muhammad Jamshed Khan, Suriya Kumari Ramiah, and Saminathan Mookiah; *investigation*: Suriya Kumari Ramiah, Muhammad Jamshed Khan, and Yashini Subramaniam; *data curation*: Suriya Kumari Ramiah and Muhammad Jamshed Khan; *writing-review and editing*: Suriya Kumari Ramiah, Muhammad Jamshed Khan, and Saminathan Mookiah. All authors read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declared that there was no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References

Ahmad, R.S., Imran, A., and Hussain, M.B. 2018. Nutritional composition of meat. In: Arshad M.S. (Ed.) Meat science and nutrition. IntechOpen Limited, London, 61(10.5772), pp. 61–75. https://doi.org/10.5772/intechopen.77045

Ali, S., Zhang, W., Rajput, N., Khan, M.A., Li, C.B. and Zhou, G.H. 2015. Effect of multiple freeze-thaw cycles on the quality of chicken breast meat. Food Chemistry 173: 808–814. https://doi.org/10.1016/j.foodchem.2014.09.095

Barbaro, A. 2022. Thinking about the future of food safety—a foresight report. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. Available at: https://www.fao.org/3/cb8667en/cb8667en.pdf (Accessed 1 April 2024).

Bennato, F., Luca, A. Di, Martino, C., Ianni, A., Marone, E., Grotta, L. and Martino, G. 2020. Influence of grape pomace intake on nutritional value, lipid oxidation and volatile profile of poultry meat. Foods 9: 508. https://doi.org/10.3390/foods9040508

Choe, J., Min, J.S., Lee, S.O., Khan, M.I. and Yim, D.G. 2018. Influence of cooking , storage period , and re-heating on production of cholesterol oxides in chicken meat. Korean Journal Food Science of Animal Resources 38(3): 433–441.

Derewiaka, D. and Lorenzo, J.M. 2022. Cholesterol and cholesterol oxidation products (COPs). In: Food lipids. Academic Press, Cambridge, MA, pp. 173–205.

Echegoyen Y., Nerín C.2013. Nanoparticle release from nano-silver antimicrobial food containers. Food Chemical Toxicology 62:16–22. https://doi.org/10.1016/j.fct.2013.08.014

Gallocchio, F., Cibin V., Biancotto, G., Roccato, A., Muzzolon, O., Carmen, L., et al. 2016. Testing nano-silver food packaging to evaluate silver migration and food spoilage bacteria on chicken meat. Food Additive and Contaminants. Part A Chemistry, Analysis, Control, Exposure & Risk Assessment 33:1063–1071. https://doi.org/10.1080/19440049.2016.1179794

Hashari, S.Z., Rahim, A.A., Meng, G.Y. and Ramiah, S.K. 2020. Quantification of cooking method effect on COP content in meat types using triple quadrupole GC-MS/MS. Molecules 25(21), 4978. https://doi.org/10.3390/molecules25214978

Khan, M.J., Kumari, S., Selamat, J., Shameli, K. and Sazili, A.Q. 2020. Reducing meat perishability through pullulan active packaging. Journal of Food Quality, 2020: 8880977. https://doi.org/10.1155/2020/8880977

Khan, M.J., Kumari, S., Shameli, K., Selamat, J. and Sazili, A.Q. 2019. Green synthesis and characterization of pullulan-mediated silver nanoparticles through ultraviolet irradiation. Materials 12(2382): 1–12. https://doi.org/doi:10.3390/ma12152382.

Khan, M.I., Min, J.-S., Lee, S.-O., Yim, D.G., Seol, K.-H., Lee, M., et al. 2015. Cooking, storage, and reheating effect on the formation of cholesterol oxidation products in processed meat products. Lipids Health Diseases 14(1): 89. https://doi.org/10.1186/s12944-015-0091-5

Khan, M.J., Ramiah, S.K., Selamat, J., Sazili, A.Q. and Mookiah, S. 2022. Utilisation of pullulan active packaging incorporated with curcumin and pullulan-mediated silver nanoparticles to maintain the quality and shelf life of broiler meat. Italian Journal of Animal Science 21(1): 244–262. https://doi.org/10.1080/1828051X.2021.2012285

Khan, M.J., Shameli, K., Sazili, A., Selamat, J. and Kumari, S. 2019. Rapid green synthesis and characterization of silver nanoparticles arbitrated by curcumin in an alkaline medium. Molecules 24(719): 1–12. https://doi.org/10.3390/molecules24040719

Li, L.H., Dutkiewicz, E.P., Huang, Y.C., Zhou, H.B. and Hsu, C.C. 2019. Analytical methods for cholesterol quantification. Journal of Food and Drug Analysis 27(2): 375–386. https://doi.org/10.1016/j.jfda.2018.09.001

Liu, Y., Liu, Y., Han, K., Cai, Y., Ma, M., Tong, Q., et al. 2019. Effect of nano-TiO_2 on the physical, mechanical and optical properties of pullulan film. Carbohydrate Polymers 218: 95–102. https://doi.org/10.1016/J.CARBPOL.2019.04.073

Macho-González, A., Bastida, S., Garcimartín, A., López-Oliva, M., González, P., Benedí, J., et al. 2021. Functional meat products as

oxidative stress modulators: A review. Advances in Nutrition 12(4): 1514–1539. https://doi.org/10.1093/advances/nmaa182

Manzoor, S., Masoodi, F.A., Rashid, R., Naqash, F. and Ahmad, M. 2022. Oleogels for the development of healthy meat products: a review. Applied Food Research 2(2): 100212. https://doi.org/10.1016/j.afres.2022.100212

Milićević D, Vranić D, Mašić Z, Parunović N, Trbović D, Nedeljković-Trailović J., et al. 2014. The role of total fats, saturated/unsaturated fatty acids and cholesterol content in chicken meat as cardiovascular risk factors. Lipids Health Diseases 13(1): 42. https://doi.org/10.1186/1476-511X-13–42

Min, J.S., Lee, S.O., Khan, M.I., Yim, D.G., Seol, K.H., Lee, M., et al. 2015. Monitoring the formation of cholesterol oxidation products in model systems using response surface methodology. Lipids in Health and disease, 14: 77. https://doi.org/10.1186/s12944-015-0074-6

Ming-Min, W. and Ismail-Fitry, M.R. 2023. Physicochemical, rheological and microstructural properties of chicken meat emulsion with the addition of Chinese yam (Dioscorea polystachya) and arrowroot (Maranta arundinacea) as meat substitutes. Future Foods 7: 100221. https://doi.org/10.1016/j.fufo.2023.100221

Mir, N.A., Rafi, A., Kumar, F., Singh, V. and Shukla, V. 2017. Determinants of broiler chicken meat quality and factors affecting them : a review. Journal of Food Science Technology 54(10): 2997–3009. https://doi.org/10.1007/s13197-017-2789-z

Mohammed, A.E., Bazargani-Gilani, A.B. and Obaid Hasson, S. 2023. Comparison of green and synthetic silver nanoparticles in zein-based edible films: shelf-life study of cold-stored Turkey breasts. Food Science & Nutrition 11(11): 7352–7363. https://doi.org/10.1002/fsn3.3661

Morsy, M.K., Khalaf, H.H., Sharoba, A.M., El-Tanahi, H.H. and Cutter, C.N. 2014. Incorporation of essential oils and nanoparticles in pullulan films to control foodborne pathogens on meat and poultry products. Journal of Food Science 79(4): M675–M684. https://doi.org/10.1111/1750-3841.12400

Mousavi Khaneghah, A., Hashemi, S.M. B. and Limbo, S. 2018. Antimicrobial agents and packaging systems in antimicrobial active food packaging: an overview of approaches and interactions. Food and Bioproducts Processing 111: 1–19. https://doi.org/10.1016/j.fbp.2018.05.001

Nadia, H. 2019. Cholesterol oxidation products (COPs) in ruminant meat : a biological and pathological approach : a review. Asian Journal of Dairy and Food Research 38(3): 191–202. https://doi.org/10.18805/ajdfr.DR-134

Noor, S., Bhat, Z.F., Kumar, S. and Mudiyanselage, R.J. 2018. Preservative effect of Asparagus racemosus : a novel additive for bioactive edible films for improved lipid oxidative stability and storage quality of meat products. Meat Science 139(1): 207–212. https://doi.org/10.1016/j.meatsci.2018.02.001

Rahim, A.A., Fahmi, M.I., Hashari, S.Z. and Thema Juhana, M.J. 2012. Detection of cholesterol oxidation products (COPs) in raw and chilled storage of chicken sausages formulated with chicken fat and red palm mid fraction. World Applied Sciences Journal 17: 51–56.

Rai, M., Wypij, M., Ingle, A.P., Trzcińska-Wencel, J. and Golińska, P. 2021. Emerging trends in pullulan-based antimicrobial systems for various applications. International Journal of Molecular Sciences 22(24): 13596. https://doi.org/10.3390/ijms222413596

Ramiah, S.K., Meng, G.Y. and Ebrahimi, M. 2014. Dietary conjugated linoleic acid alters oxidative stability and alleviates plasma cholesterol content in meat of broiler chickens. The Scientific World Journal, 2014: 949324. https://doi.org/http://dx.doi.org/10.1155/2014/949324

Rather, S.A., Masoodi, F.A., Rather, J.A., Gani, A., Wani, S.M., Ganaie, T.A., et al. 2021. Impact of thermal processing and storage on fatty acid composition and cholesterol oxidation of canned traditional low-fat meat product of India. Food Science and Technology (LWT) 139: 110503. https://doi.org/10.1016/j.lwt.2020.110503

Serra, A., Conte, G., Cappucci, A., Casarosa, L., Serra, A., Conte, G., et al. 2014. Cholesterol and fatty acids oxidation in meat from three muscles of massese suckling lambs slaughtered at different weights. Italian Journal of Animal Science 13(3275): 648–652. https://doi.org/10.4081/ijas.2014.3275

Synowiec, A., Gniewosz, M., Kraśniewska, K., Przybył, J.L., Bączek, K. and Węglarz, Z. 2014. Antimicrobial and antioxidant properties of pullulan film containing sweet basil extract and an evaluation of coating effectiveness in the prolongation of the shelf life of apples stored in refrigeration conditions. Innovative Food Science and Emerging Technologies 23: 171–181. https://doi.org/10.1016/j.ifset.2014.03.006

Trinetta, V. and Cutter, C.N. 2016. Pullulan: a suitable biopolymer for antimicrobial food packaging applications. In: Barros-Velázquez, J. (ed.) Antimicrobial food packaging. Elsevier, Penn State, State College, PA, pp. 385–397. https://doi.org/10.1016/B978-0-12-800723-5.00030-9

Winiarska-Mieczan, A., Kwiecien, M., Grela, E.R., Tomaszewska, E. and Klebaniuk, R. 2016. The chemical composition and sensory properties of raw , cooked and grilled thigh meat of broiler chickens fed with Fe-Gly chelate. Journal of Food Science and Technology 53(10): 3825–3833. https://doi.org/10.1007/s13197-016-2374-x

Xu, L. and Porter, N.A. 2015. Free radical oxidation of cholesterol and its precursors: implications in cholesterol biosynthesis disorders. Free Radical Research 49(7): 835–849. https://doi.org/10.3109/10715762.2014.985219

Yao, Y., Liu, Y., Li, C., Huang, X., Zhang, X., Deng, P., et al. 2018. Effects of rosemary extract supplementation in feed on growth performance, meat quality, serum biochemistry, antioxidant capacity, and immune function of meat ducks. Poultry Science 102(2): 10235. https://doi.org/10.1016/j.psj.2022.102357

Zerbinati, C. and Iuliano, L. 2017. Cholesterol and related sterols autoxidation. Free Radical Biology and Medicine 111: 151–155. https://doi.org/10.1016/j.freeradbiomed.2017.04.013