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, angio-toxic, 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 \textit{et al.}, 2015; Li \textit{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 \textit{et al.}, 2018; Serra \textit{et al.}, 2014). Many scientists reported a strong relationship between food safety, human health, and intake of fat contents of meat, that is, polysaturated fatty acids (PUFAs) (Barbaro, 2022; Khan \textit{et al.}, 2015; Serra \textit{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 $\Delta$-5 in the chemical configuration of PUFA intensifies oxidation process, resulting in COPs (Nadia, 2019).

The oxidation process in all 70 known types of cholesterol 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, carcinogetic, angitoxic, 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; Miličević 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 Ecchegoyen 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 ±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). 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.

<table>
<thead>
<tr>
<th>Table 1. Meat samples (Pectoralis major) treated with pullulan active packaging at different storage periods.</th>
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<tbody>
<tr>
<td>C0</td>
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<td>C14</td>
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<td>T14</td>
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</tbody>
</table>

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-cholestan, 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-cholestan, 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-cholestan 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,
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 ≤ 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 ≤ 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.
Quality Assurance and Safety of Crops & Foods 16 (2) 85

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

<table>
<thead>
<tr>
<th>Cholesterol oxidation products (COPs)</th>
<th>Duration of poultry meat storage</th>
<th>COP's mean values</th>
<th>ANOVA</th>
<th>Coefficient of variation (R)</th>
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<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7th</td>
<td>Day 14th</td>
<td>F value</td>
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<tr>
<td>A-epoxy (ppm)</td>
<td>11.30 ± 7.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.20 ± 3.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.72 ± 3.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.70 ± 3.18&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>B-epoxy (ppm)</td>
<td>0.30 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.593 ± 0.21&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>A-cholestane (ppm)</td>
<td>0.30 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.31 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.03 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.634 ± 0.08&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cholesterol (ppm)</td>
<td>18.24 ± 23.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.27 ± 10.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.37 ± 10.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.60 ± 11.13&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>7-keto (ppm)</td>
<td>1.60 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.11 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.81 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.927 ± 0.18&lt;sup&gt;ns&lt;/sup&gt;</td>
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<tr>
<td>25-OH (ppm)</td>
<td>3.28 ± 5.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.16 ± 2.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.60 ± 3.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.657 ± 2.50&lt;sup&gt;ns&lt;/sup&gt;</td>
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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 pullulan active packaging treatments.

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 cholesterol present in meat (Min et al., 2015). A similar pattern was observed in the present study. Pullulan active packaging with treatments T1 (0.29±0.17; 0.31±0.37) and T2 (0.28±0.18; 0.31±0.37) significantly (P < 0.05) maintained the concentrations (ppm) of A-cholestane (P ≤ 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-hydroperoxylcholesterol’ (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.

Conflicts of Interest

The authors declared that there was no conflict of interest.

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