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Shelf Life Extension of Chicken Cuts Packed under Modified Atmospheres and Edible Antimicrobial Coatings

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Abstract: Convenient cuts of poultry products are of significant interest, but they are perishable products with a short shelf life. Modified atmosphere packaging (MAP) or the application of active packaging based on edible and biodegradable coatings could extend the shelf life of perishable foods. The aim of the present work was the kinetic modelling of the effect of MAP and active coatings with antimicrobial agents on the microbial growth and shelf life of chicken cuts. Broiler chicken thighs processed with pectin-based (2% *w*/*w* aqueous solution) edible coatings enriched with 1% extract of citrus bioflavonoids (flavomix) or 0.5% glucono-δ-lactone were stored under aerobic conditions at 0, 5 and 10 °C. Untreated thighs were also stored aerobically or in MAP (42.7% O_2, 18.5% CO_2). Quality evaluation was based on the growth of spoilage bacteria (TVC, *Pseudomonas* spp., *Brochothrix thermosphacta*), pH, colour, and sensory scoring. The tested quality indices were kinetically modelled and the Arrhenius model was used for the definition of the temperature dependence of the quality loss rates. *Pseudomonas* spp. dominated spoilage at all packaging and temperature conditions. Microbial growth correlated well with sensory degradation (E_a = 80–100 kJ/mol). Glucono-δ-lactone-enriched edible coatings resulted in 2 days of shelf life extension for chicken thighs at 5 °C. MAP and active, edible coatings with citrus extract showed a similar effect on the quality deterioration rate, and thus the shelf life of chicken cuts. Based on microbial growth, the shelf life was 6–7, 11, and 13 days at 5 °C for the control, EC-glu, MAP, and EC-flav samples, respectively (limit of acceptability = 10^7 cfu/g for TVC). The results of the study show the potential for using MAP or edible, active coatings to extend the shelf life and improve the commercial value of broiler chicken cuts.

Keywords: active packaging; edible coatings; modified atmosphere packaging; poultry; shelf life; sustainable packaging; spoilage

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1. Introduction

Meat consumption has been focused on poultry during the past decades, which has in fact doubled in many countries worldwide between 2000 and 2019 [1,2]. Factors such as low price, high nutritional value due to the presence of high-quality protein, low amounts of fat, mild sensory characteristics, ease and short time of cooking, and cultural acceptability in some countries [3–5], as well as the need to fill in the gaps of the overall decreased consumption of both beef and pork meat [6,7], are a few of the most crucial aspects that lead to the abovementioned increase in poultry and mainly chicken consumption. According to FAO [8], the global production of poultry meat per year is about 127 million tons, making poultry industry the biggest meat sector worldwide, which is expected to represent 41% of all the protein from meat sources in 2030 [8,9]. However, the aforementioned increased poultry meat production and consumption has led to an approximately 3.7 to 4.2% loss of meat due to spoilage per year [10].

Fresh chicken meat, and especially chicken cuts, is a sensitive food product due to its high concentration of nutrients in the flesh, high levels of water activity, and near-neutral pH, which are the main parameters resulting in the fast growth of spoilage and pathogen microorganisms and, consequently, in a fast loss of freshness and, therefore, a short shelf life [11–14]. The majority of the microorganisms responsible for the spoilage of poultry meat are mostly found on its surface. Microorganisms such as *Pseudomonas* spp., Enterobacteriaceae, and lactobacilli, which are the predominant bacteria, as well as biochemical reactions, result in changes such as proteolysis, amino acid degradation and, most importantly, lipid oxidation which, at the latter stages of the storage of poultry meat, induce the development of off-flavours and slime [15,16] and result in lowering the quality and shortening the commercial shelf life of the product [17–19]. One of the most important parameters to take into consideration is the temperature; therefore, refrigeration of chicken meat is very important for the extension of the shelf life by delaying microbial growth and as a result spoilage. Although refrigeration is an absolutely necessary preservation technique to retain the microbial status and high quality of fresh poultry products, it cannot alone assure an extended shelf life. Inappropriate storage conditions, such as temperature fluctuations, inadequate packaging, and long shelf life, may lead to microbial, physicochemical, and undesirable sensory changes [20]. Therefore, preventing or inhibiting the quality deterioration of chicken meat stored under refrigeration might be beneficial for the sustainability of the final product [21].

Among the approaches applied during meat processing, for the prolongation of the shelf life and maintenance of meat products' quality, is the packaging. Food packaging plays an important role in the packaging industry sector and innovations in this field have been motivated by consumer demands, as well as by environmental issues and legislation. Modified atmosphere packaging (MAP) or the application of active packaging based on edible films may significantly extend the shelf life of perishable poultry cuts. MAP has been recently increasingly applied for the packaging of foods of animal origin [22] and has become the most ubiquitous method for retail packaging of fresh and processed meat as an alternative nonthermal food preservation system [23]. Its efficiency in significantly extending the shelf life of meat relies on carbon dioxide, which is the main gas in the package and is well known for its antibacterial properties [24]. Although MAP, in addition to its several advantages, has been characterized by a number of shortcomings, in the EU, the application of MAP alongside the appropriate management of the cold chain is the most frequently preventing strategy used against microbial growth. However, the limitations that still exist make it imperative for other strategies to be used as well.

Active packaging, which could comprise one of the aforementioned strategies, is a type of food packaging in which different bioactive compounds are incorporated in low concentrations, so as to maintain food quality and simultaneously improve shelf life [25–27]. Generally, active packaging systems have been designed to release active substances, such as antimicrobials, antioxidants, oxygen scavengers, etc., either into the headspace or product or to remove substances from the food or headspace of the packaging [28,29]. One of the main advantages of the coatings is that they remain on the food product and, therefore, protect it from contamination during storage and handling [30]. Among the different types of active packaging based on edible and biodegradable coatings is the antimicrobial coating, which is increasingly being used for preventing food contamination due to its efficacy and continuous protection of the product. The embedded antimicrobial agents that are released from the packaging can interact with biological molecules and, therefore, delay microbial growth, targeting both spoilage bacteria and food pathogens [31,32]. The antimicrobial packaging systems contain antimicrobial agents, such as, e.g., essential oils [33,34], polycations [35], chitosan [36], pectin [37,38], and nanoparticles [39], in order to delay or inhibit microbial growth or subsequent microbial contamination during storage and transportation [40]. Edible coatings may be produced using protein, polysaccharides, lipids, and the combined use of edible compounds, and can be applied by either spraying or an immersion step. Dipping techniques form films (thick membranes) over the surface

of the product by directly immersing the food into the aqueous coating formulation and subsequent air-drying of the film.

Pectin is an important structural polysaccharide of several higher plant cells which allows primary cell wall extension and plant growth. Pectin may be extracted and used as an anionic biopolymer or a soluble in water [38]. It is a plant-derived heteropolysaccharide, which is widely applied as a gelling and thickening agent in food products, such as jelly, marmalades, and confectionaries, while also acting as a main component of edible coatings in food products [41]. Polysaccharide-based films show appropriate mechanical properties and may act as an effective barrier against gases, leading to shelf life extension, without resulting in anaerobic conditions inside the food product [42]. Due to its cost efficiency, high availability, biodegradability, and feasibility to film production, either alone or in the combined application of other polymeric matrices, pectin-based edible films may be evaluated as an environmentally friendly and appropriate alternative to conventional, petroleum-based plastics. Natural antibacterial compounds, such as citrus bioflavonoids, are effective against several foodborne pathogens and spoilage bacteria. Citrus fruits have been reported as rich in flavonoid compounds, such as hesperidin, hesperetin, naringin, naringenin, diosmin, quercetin, rutin, nobiletin, and tangeretin [43]. Glucono-δ-lactone (GDL), which can be also used as an active component in edible coatings for foods, is a derivate of glucose and is a ring-shaped molecule. GDL has six carbon (C) atoms and an OH group which is attached to any C. GDL is a naturally occurring food ingredient, which is widely known as an acidity regulator in foodstuffs. It can be also incorporated into food formulations in order to delay the deterioration of food products and prevent discolouration.

Convenient cuts of poultry products are of significant interest but are perishable products with shorter shelf life than a whole chicken carcass. The growing demand for convenient and ready-to-cook, sliced, and pre-packed poultry products requires the proper handling of the food to prevent microbial contamination and loss of freshness. Shelf life extension of chicken cuts has been evaluated by appropriate monitoring of the cold chain and predictive modelling [2], the application of alternative decontamination technologies [11], low-temperature storage, and MAP for chicken fillets [12]. MAP and antimicrobial active packaging based on edible films could be applied in order to prolong the shelf life of fresh poultry cuts. An edible coating prepared by whey protein has been applied for the preservation of chicken breast fillets, using oregano and clove essential oil as active components for shelf life prolongation [14]. Oregano and thyme essential oils have been also incorporated into a chitosan-based edible coating for chicken meat [44]. Although essential oils can be applied as effective antimicrobial components for the shelf life extension of perishable foods, they affect significantly the sensory attributes of food (mainly odour and taste). For this reason, extracts of spices have been tested as alternative antimicrobial systems in combination with MAP for chicken meat [20]. Pectin is an effective compound currently used in edible coatings developed for fruits [41]. The objective of the study was the kinetic modelling of the effect of MAP and an active coating based on pectin with antimicrobial agents other than essential oils on the spoilage process and shelf life of chicken cuts.

2. Materials and Methods

2.1. Sample Preparation and Storage

Broiler chicken thighs (weight 400 ± 50 g each, 600 thighs were tested in total corresponding to 150 thighs per experimental group) were provided by a local producer (Evvoia, Greece) and received at the Laboratory of Food Chemistry and Technology (NTUA) within one day after cutting and packaging. Upon receipt, samples were immersed into a film-forming solution for 10 min at 5 °C for the application of the edible coating on their surface. The tested edible coatings were pectin (2% *w*/*w* aqueous solution) enriched with 1% organic extract of citrus bioflavonoids (Flavomix 14PO, Polypan Group, Moschato, Greece, coded as flavomix) or 0.5% glucono-δ-lactone (g-δ-l), and coated samples were

subsequently stored aerobically at isothermal conditions (0, 5 and 10 °C). Additionally, untreated thighs were also stored aerobically (control) or under MAP (42.7% O_2, 18.5% CO_2) in high-density polyethylene (HDPE) pouches (Boss NT42N, Bad Homburg, Germany, 2 thighs per package). All samples were stored in high-precision (±0.2 °C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan). The temperature in the incubators was continuously recorded using electronic, programmable miniature dataloggers (COX TRACER®, Belmont, NC, USA). Sampling took place in appropriate time intervals for the efficient kinetic modelling of quality deterioration.

2.2. Shelf Life Evaluation

Quality evaluation of the chicken samples was based on microbial growth (TVC, *Pseudomonas* spp., *Brochothrix thermosphacta*), pH, colour, and sensory scoring.

For the evaluation of microbial load, 10 g of chicken flesh (including the skin) was transferred to a sterile stomacher bag with a 90 mL sterilised Ringer solution (Merck, Darmstadt, Germany). Homogenisation was achieved using a Stomacher (BagMixer® Interscience, Saint Nom la Bretêche, France) for 60 s. Samples (0.1 mL) of 10-fold serial dilutions of meat homogenates were spread on the surface of the appropriate media in Petri dishes for the enumeration of different spoilage bacteria. The microbial load was expressed as the average log CFU/g. TVC was enumerated on a plate count agar (PCA, Merck, Darmstadt, Germany) after incubation at 25 °C for 72 h, whereas *Pseudomonas* spp. were enumerated on a Cetrimide agar (CFC, Merck, Darmstadt, Germany) after incubation at 25 °C for 48 h. *Brochothrix thermosphacta* was enumerated on an STAA agar (CM 881, Oxoid, Cambridge, UK) supplemented with SR 151 (Oxoid) and incubation at 25 °C for 48 h. Two replicates of at least three appropriate dilutions were enumerated for each sampling point and growth medium.

Quantification of the colour of the tested samples was made using a CR-Minolta Chromameter® (Minolta CR-200, Osaka, Japan) with an 8 mm measuring diameter, using the CIE L*, a*, b* colour scale (CIE 1978). The instrument was standardised under the "C" illuminant condition according to the CIE using a standard white reference tile (calibration plate CR-200, L = 97.50, a = −0.31, b = −3.83). The overall colour change, ΔE, was calculated by $\Delta E = [(L - L_o)^2 + (a - a_o)^2 + (b - b_o)^2]^{1/2}$. Colour measurement of chicken meat was carried out at three points of five specimens.

The pH value of the tested samples was measured in the diluted meat in Ringer's solution at a ratio of 1:10 during the shelf life experiment (pH-meter AMEL 338, Italy) (n = 3). pH measurement was implemented in the homogenized meat sample into the Ringer solution after the sampling for microbiological analysis.

The sensory parameters of raw chicken samples were evaluated by a sensory panel of 6. The sensory panel participated in a preliminary training session, where the overall process and point scale were introduced by introducing the reference samples corresponding to different quality levels of the tested food product. Sensory evaluation was implemented on uncooked chicken thighs, considering the odour/off-odour and the visual appearance/colour. The samples were displayed to the evaluators on 3-digit coded white paper plates. The appearance and odour of chicken samples were rated using a 1–9 hedonic scale (9 = like extremely and 1 = dislike extremely). Panellists were also asked to evaluate the overall impression and acceptability. A sensory score of 5 was taken as the average scoring for minimum acceptability, indicating the end of shelf life. Scores given for the sensory attributes were mathematically modelled by apparent zero-order equations:

$$s = s_o - k \cdot t,$$

where s and s_o are the sensory scores at time t and zero, respectively, and k is the apparent rate of quality deterioration based on sensory scoring (d^{-1}).

The overall experimental design is demonstrated in Table 1.

Table 1. Description of the experimental design.

Control (Total 150 Samples)		
0 °C	**5 °C**	**10 °C**
a. Microbial enumeration + pH: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples	a. Microbial enumeration + pH: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples	a. Microbial enumeration + pH: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples
MAP (Total 150 Samples)		
a. Microbial enumeration, pH, gas analysis: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples	a. Microbial enumeration, pH, gas analysis: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples	a. Microbial enumeration, pH, gas analysis: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples
EC-flav (Total 150 Samples)		
a. Microbial enumeration + pH: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples	a. Microbial enumeration + pH: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples	a. Microbial enumeration + pH: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples
EC-glu (Total 150 Samples)		
a. Microbial enumeration + pH: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples	a. Microbial enumeration + pH: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples	a. Microbial enumeration + pH: 20 samples b. Colour: 5 samples c. Sensory evaluation: 25 samples

2.3. Data Analysis

The microbial growth was modelled by fitting the experimental data with the Baranyi Growth Model [45]. For curve fitting, the macros included in DMFit software (IFR, Institute of Food Research, Reading, UK) were used (available at http://www.combase.cc/index.php/en/, accessed on 8 August 2022). The kinetic parameters of microbial growth, i.e., rate (k) and lag phase (λ), were determined.

Temperature dependence of microbial growth and histamine formation were modelled using the Arrhenius equation (Equation (1)):

$$\ln k = \ln k_{ref} - \left(\frac{E_a}{R}\right)\left[\frac{1}{T} - \frac{1}{T_{ref}}\right] \quad (1)$$

where k_{ref} (in d^{-1}) is the respective rate constant at a reference temperature, T_{ref} (in this case 4 °C) and T are the storage temperatures (in K), E_a is the activation energy of the

specific action (in J/mol), and R is the universal gas constant. The E_a values were calculated based on the slope of the Arrhenius plots vs. $(1/T_{ref} - 1/T)$ linear regression [46].

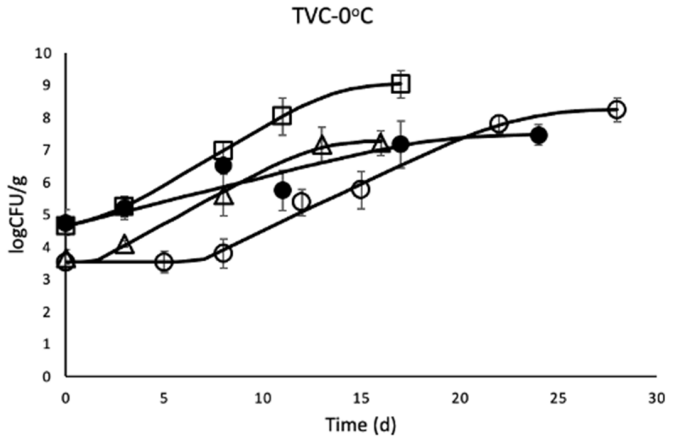
2.4. Statistical Analysis

Analysis of variance (two-factor ANOVA) at a significance level of 95% was applied for the analysis of the studied quality degradation rates for all sample series, i.e., control, MAP, and samples coated with edible antimicrobial materials during storage at refrigerated conditions. Significant differences were determined based on Duncan's multiple range test (a = 0.05) (STATISTICA® 7.0, StatSoft Inc., Tulsa, OK, USA). Multivariate statistical analysis (principal component analysis, PCA) was used to define the correlations between the tested packaging types and the quality indices of fresh chicken cuts during isothermal storage at 0–10 °C (XLSTAT 2023.1.1, www://www.xlstat.com/en, accessed on 5 January 2023).

3. Results and Discussion

3.1. Microbial Enumeration of Chicken Thighs

Microbial counts of all bacteria tested in the present study increased with storage time at all temperatures tested (0, 5 and 10 °C) ($p < 0.05$). The total viable count (TVC) is a microbiological index that estimates the overall concentration of microorganisms in a sample and helps to define the hygiene level and remaining shelf life of food [47]. In the present study, initial microbial counts in the control samples were 4.6, 3.9, and 2.5 logcfu/g for TVC, *Pseudomonas* spp., and *Brochothrix thermosphacta*, respectively. Similar results concerning the initial values of TVCs and *Pseudomonas* spp. Were reported by Giatrakou et al. [44] and Ntzimani et al. [48], who reported initial values of approximately 4–5 log cfu/g for these microorganisms in fresh and pre-cooked chicken cuts stored aerobically at 4 °C, respectively, whereas initial counts noted in the present study for *Br. Thermosphacta* and *Enterobacteriaceae* were lower as compared to the aforementioned studies [44,48–50]. The TVC values increased to 9.0, 7.5, 8.2, and 7.2 log CFU/g on the last day of storage for each treatment (day 17, 24, 28, and 16) for the control, MAP, EC-flav and, EC-glu, respectively, which exceeded the upper limit set for microbiological acceptability (7 logcfu/g) after approximately 8, 15, 19, and 12 days of storage at 0 °C [12,18,24,47,48,51]. In the case where the chicken cuts were stored at higher temperatures (5 and 10 °C), the abovementioned limit was reached at a shorter storage time, resulting in shorter shelf life as compared to that of samples stored at 0 °C (Figure 1a–c). Results were in line with the respective results reported by Yimenu et al. [50], who reported similar values of TVCs. They reported that values of TVC increased to 7.3, 8.0, 8.1, and 8.7 log CFU/g after approximately 9, 6, 3, and 2 days when chicken breasts were stored at 0, 5, 10, and 15 °C, respectively. Similar results were reported by Ghollasi-Mood et al. [2] and Ntzimani et al. [48]. Faster microbiological growth was recorded by the increase in storage temperature and time ($p < 0.05$), as also reported by other researchers [48,49,52]. Additionally, these microorganisms were found to be the initial microflora for the chicken cuts and were also stored under MAP conditions at all temperatures tested, which is in accordance with the results of previous studies [12,13]. Microbial growth was significantly inhibited in the chicken samples coated with the edible packaging materials and MA-packaged chicken at all storage temperatures (Figures 1 and 2), similar to growth rates in the EC-flav and EC-glu samples ($p < 0.05$). The application of MAP resulted in a shelf life extension of approximately 7 and 4–5 days when the chicken cuts were stored at 0 and 5 °C, respectively. A similar effect of MAP was reported by Balamatsia et al. [23], who stated a shelf life extension of 4 days of MA-packed chicken breast fillets stored at 4 °C and Patsias et al. [12], who also noted a respective shelf life extension of chicken fillets. Fernández-Pan et al. [14] also reported a 7-day shelf life prolongation of the MA-packed samples at refrigerated storage (4 °C).



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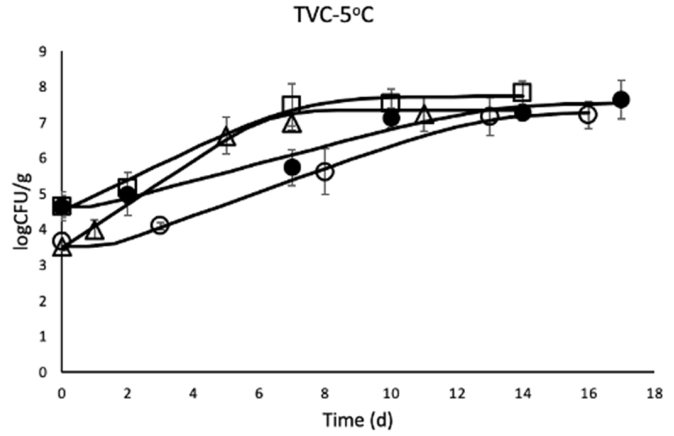

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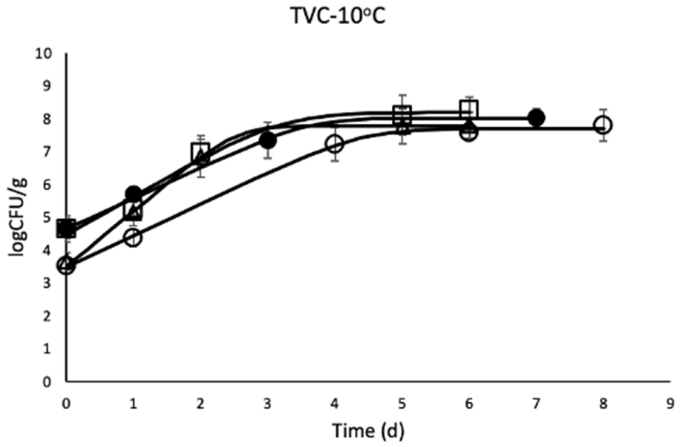
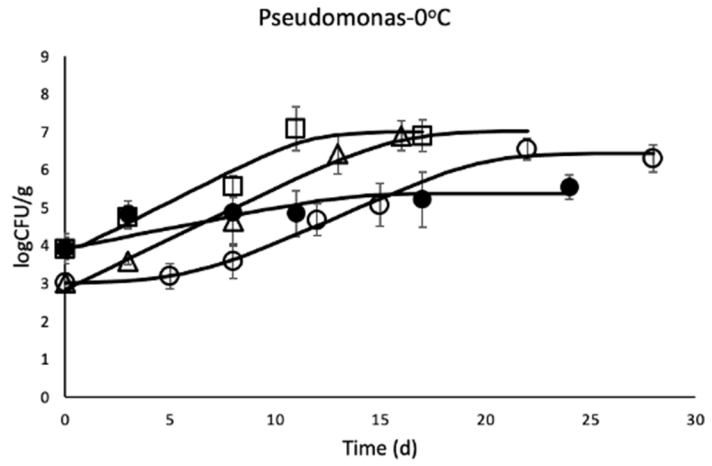


(**c**)

Figure 1. Enumeration of TVC in chicken stored at (**a**) 0 °C, (**b**) 5 °C, and (**c**) 10 °C (□ control, ● MAP, ○ EC-flav, △ EC-glu).



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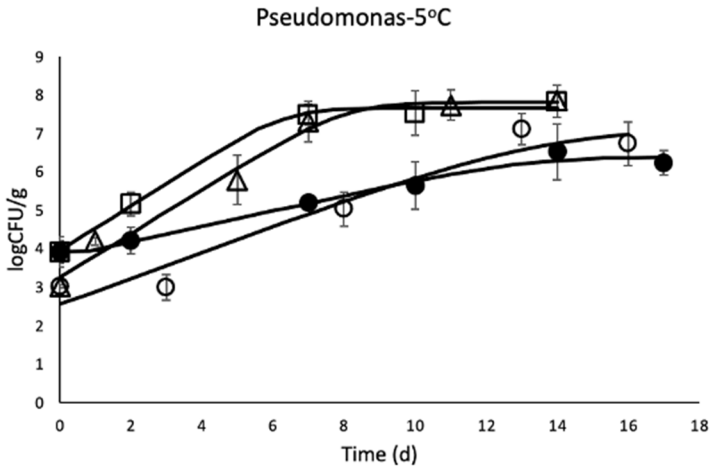

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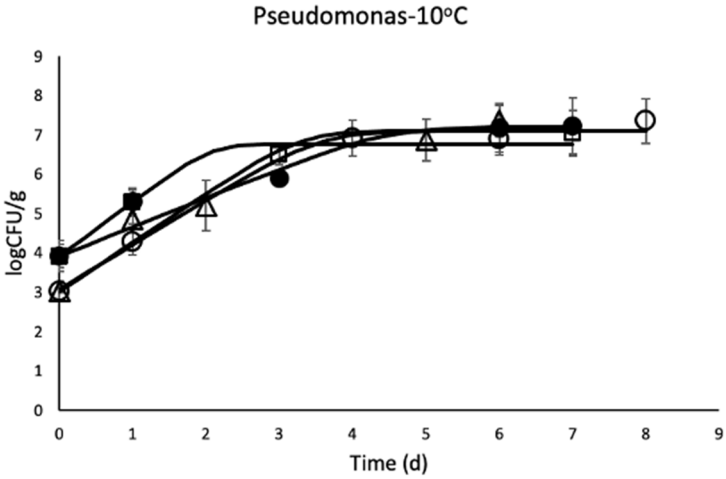


(**c**)

Figure 2. Enumeration of *Pseudomonas* spp. in chicken stored at (**a**) 0 °C, (**b**) 5 °C, and (**c**) 10 °C (□ control, ● MAP, ○ EC-flav, △ EC-glu).

The application of the antimicrobial edible coating resulted in approximately 1 logcfu/g reduction in the initial TVC, showing an important effect on the microorganisms responsible for the spoilage of the chicken cuts. Similar outcomes have been noted by Yehia et al. [47],

where the TVC of the samples treated using 2% citrus extract revealed a reduction by 2.5 and 2.0 log units compared to the untreated samples, indicating the preservative effect of the citrus extract on the bacteria. Additionally, according to Fernández-Pan et al. [14], the high effectiveness of edible films against the autochthonous spoilage microorganisms in fresh chicken breasts applied during storage at different temperatures was also reported. *Pseudomonas* spp. dominated spoilage at all packaging and temperature conditions. Counts of *Br. thermosphacta* during storage of the chicken cuts at 0 °C were significantly lower ($p < 0.05$) than counts during storage at 5 and 10 °C, which was in agreement with the respective results noted by other researchers [49]. Microbial growth rates were estimated by fitting the Baranyi Growth Model to the experimental data (Table 2).

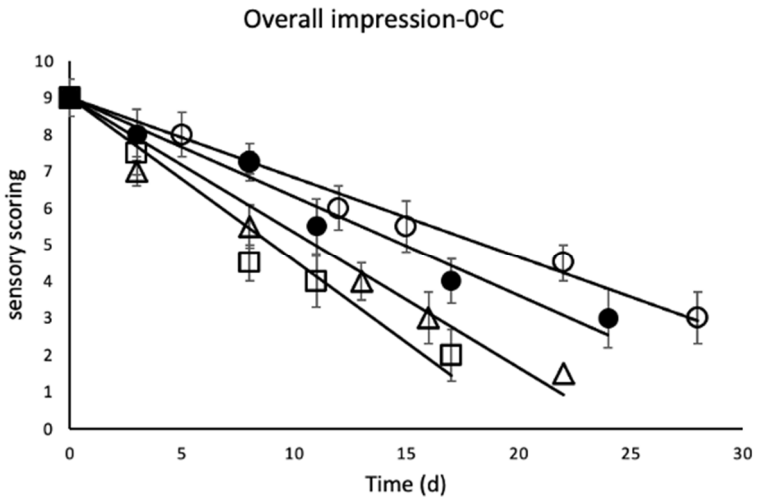
Table 2. Microbial growth rates (k in d^{-1}) for chicken thighs stored at 0, 5, and 10°C, and activation energy (E_a) values calculated by the Arrhenius equation.

	0 °C	**5 °C**	**10 °C**	**E_a (kJ/mol)**
		Control		
k_{TVC}	0.3655 ± 0.0045 ^{a3}	0.8835 ± 0.1001 ^{b3}	1.7347 ± 0.0561 ^{c2}	100.2 (R^2 = 0.9956)
$k_{Pseudomonas}$	0.2796 ± 0.0906 ^{a2}	0.5811 ± 0.0828 ^{b3}	1.3686 ± 0.2232 ^{c2}	102.1 (R^2 = 0.9969)
$k_{B.thermosphacta}$	0.3224 ± 0.0878 ^{a2}	0.6615 ± 0.0368 ^{b4}	0.8485 ± 0.0089 ^{c3}	62.4 (R^2 = 0.9325)
		MAP		
k_{TVC}	0.1511 ± 0.0607 ^{a1}	0.2424 ± 0.0537 ^{a1}	0.9304 ± 0.0974 ^{b1}	116.5 (R^2 = 0.9223)
$k_{Pseudomonas}$	0.1080 ± 0.0722 ^{a1}	0.1996 ± 0.0295 ^{a1}	0.7378 ± 0.3447 ^{b1}	123.3 (R^2 = 0.9542)
$k_{B.thermosphacta}$	0.2024 ± 0.0385 ^{a1}	0.2176 ± 0.0532 ^{a1}	0.7135 ± 0.0239 ^{b2}	80.6 (R^2 = 0.9245)
		EC-flav		
k_{TVC}	0.2911 ± 0.0244 ^{a2}	0.6183 ± 0.0715 ^{b2}	0.9642 ± 0.0531 ^{c1}	77.1 (R^2 = 0.9812)
$k_{Pseudomonas}$	0.2435 ± 0.0591 ^{a2}	0.3825 ± 0.0169 ^{b2}	0.6317 ± 0.2499 ^{c1}	98.5 (R^2 = 0.9417)
$k_{B.thermosphacta}$	0.2065 ± 0.0511 ^{a1}	0.3694 ± 0.0623 ^{b2}	0.5551 ± 0.0096 ^{c1}	63.7 (R^2 = 0.9917)
		EC-glu		
k_{TVC}	0.2833 ± 0.0256 ^{a2}	0.6605 ± 0.0236 ^{b2}	1.6833 ± 0.0342 ^{c2}	114.6 (R^2 = 0.9985)
$k_{Pseudomonas}$	0.2667 ± 0.0266 ^{a2}	0.5154 ± 0.1154 ^{b3}	1.2414 ± 0.7356 ^{c2}	98.8 (R^2 = 0.9914)
$k_{B.thermosphacta}$	0.2052 ± 0.0168 ^{a1}	0.4791 ± 0.0119 ^{b3}	0.7274 ± 0.0273 ^{c2}	81.5 (R^2 = 0.9667)

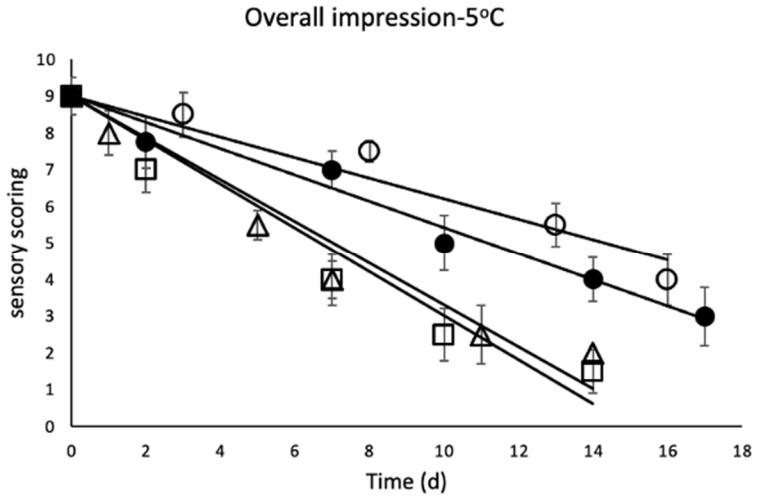
$^{a–c}$ Means with different lowercase letters in each raw indicate significant differences within the tested storage temperatures ($p < 0.05$). $^{1–4}$ Means with different lowercase numbers for each tested microorganism and storage temperature indicate significant differences within the tested preservation methods, i.e., control, MAP, EC-flav, and EC-glu ($p < 0.05$).

3.2. Sensory Evaluation of Chicken Thighs

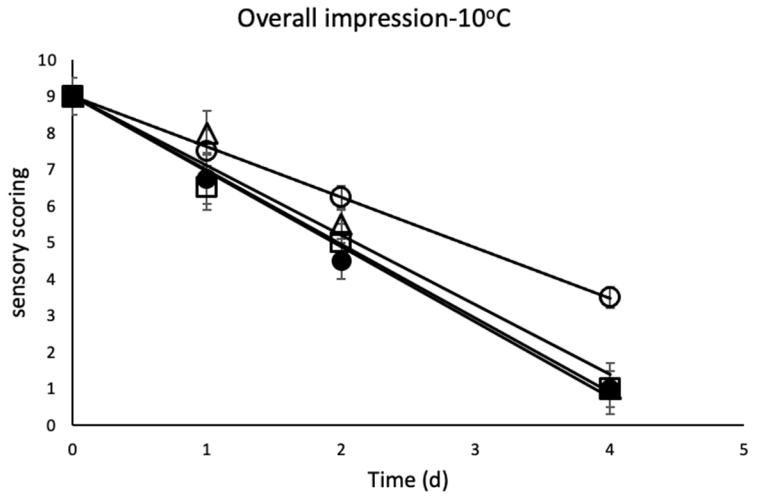
Sensory scoring was adequately described by an apparent zero-order equation $s = s_o - k \cdot t$, where s_o and s are the sensory scoring initially and at time t of storage and k is the sensory scoring reduction rate (d^{-1}), i.e., the slope of the s-t line in Figure 3. The limit of acceptability (rejection level) was scoring a 5 for the overall sensory impression. Sensory scoring decreased significantly ($p < 0.05$) with the increase in storage temperature (0, 5 and 10 °C). This was in agreement with the sensory evaluation of Yimenu et al. [50], who reported that the sensory parameters determining the sensory quality of the packaged chicken breast meat decreased, at a temperature-dependent rate, as storage time increased. At all storage temperatures tested, on day 0 of storage, the chicken cuts had an excellent appearance and odour (score 9). In general, odour attributes of chicken thighs deteriorated faster than the appearance ($p < 0.05$), indicating that for the panellists, the undesired smell of uncooked chicken cuts would be a more reliable indication of poor quality than appearance and colour, which was also reported by Katiyo et al. (2020) [5].



(**a**)



(**b**)



(**c**)

Figure 3. Sensory scoring (overall impression) of chicken stored at (**a**) 0 °C, (**b**) 5 °C, and (**c**) 10 °C (□ control, ● MAP, ○ EC-flav, △ EC-glu).

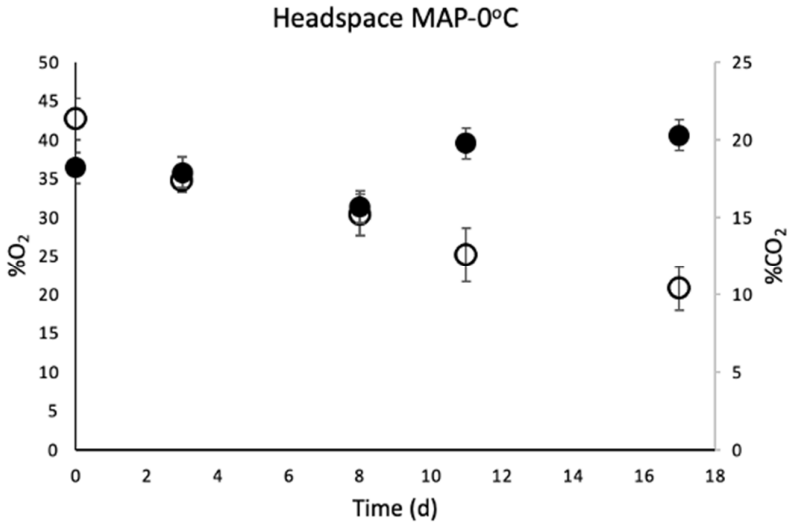
Packaging significantly affected the sensory scoring of chicken ($p < 0.05$), with the application of edible coating with flavomix (EC-flav) and packaging under MA showing the strongest preservative effect at all tested storage temperatures. The activation energy E_a of the sensory scoring reduction rates for the alternative packaging types were 79.5, 92.2, 92.4, and 82.3 kJ/mol for the control, MAP, EC-flav, and EC-glu samples, respectively. The use of edible coatings containing flavomix could also improve the nutritional and sensory attributes of the chicken cuts. The results of the sensory evaluation (Figure 3a–c) correlated well with those of the microbiological analyses (Figure 1a–c) at all storage temperatures used. The results of the present study, as far as sensory evaluation is concerned, were in accordance with the findings of other researchers [47]. The rates of the sensory degradation of all samples were higher at higher temperatures ($p < 0.05$), which was in accordance with the respective results reported by Ntzimani et al. [48].

3.3. Determination of Colour Parameters of Chicken Thighs

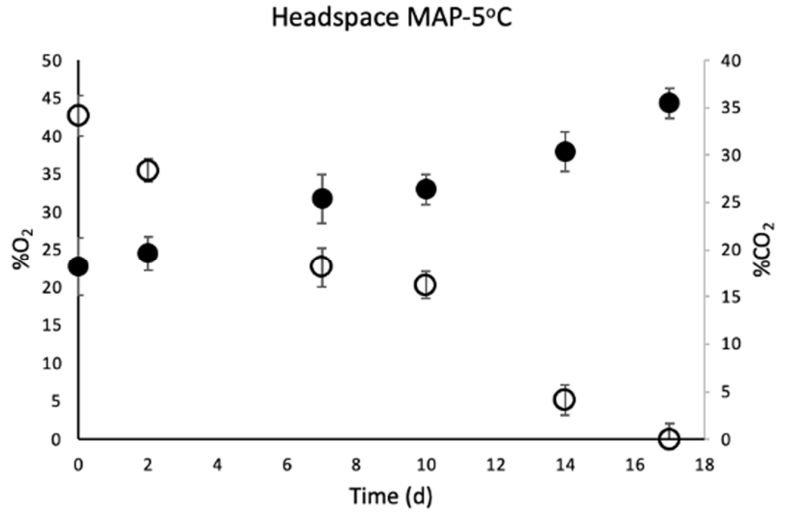
As far as the ΔE values of the chicken samples are concerned, no significant changes were observed ($p > 0.05$). Initial lightness (L-value) was 73.5 ± 4.9, 75.9 ± 3.6, 73.2 ± 2.1, and 74.6 ± 3.6 for the control, MAP, EC-flav, and EC-glu samples, respectively. The respective a-values were 4.2 ± 0.4, 4.5 ± 0.8, 5.9 ± 1.1, and 4.4 ± 0.9, while the b-values were initially 11.3 ± 3.1, 9.6 ± 0.7, 8.2 ± 1.5, and 8.7 ± 0.8 for the control, MAP, EC-flav, and EC-glu samples. Neither the storage temperature nor the packaging/treatment of the samples had an effect on the ΔE values of the chicken as compared to the control samples ($p > 0.05$). Additionally, it was observed that ΔE values were lower for the MAP, flavomix, and g-δ-l samples in comparison to the control samples stored at 0 °C, which was in line with the observations by Patsias et al. [12], who also observed that colour parameters and especially lightness was not affected by the different conditions and treatments tested in the work. Similar results have also been reported by Mexis et al. [51] for MA-packed lamb meat. Katiyo et al. (2020) reported that the chroma of chicken skin does not differ significantly between samples under different levels of spoilage (i.e., stored for different durations under refrigeration), and thus cannot be considered an adequate quality index [5].

3.4. Determination of Headspace Composition of MAP Chicken Thighs

The composition of the internal atmosphere of the MA-packaged chicken was 42.7 ± 1.5% O_2, 18.5 ± 0.5% CO_2 και 38.8 ± 0.7% N_2, whereas the rest of the samples were aerobically packaged. The percentage of O_2 was reduced in all samples, regardless of the storage temperature (Figure 4). During the storage of MAP chicken samples at all temperatures, the % CO_2 increased due to the metabolism of the microorganisms [53]. As indicated in Figure 4a–c, the mutual effect of oxygen deficiency after the first days of storage at all tested temperatures and the bacteriostatic activity of CO_2 significantly delayed microbial growth, as discussed in Section 3.1.



(**a**)



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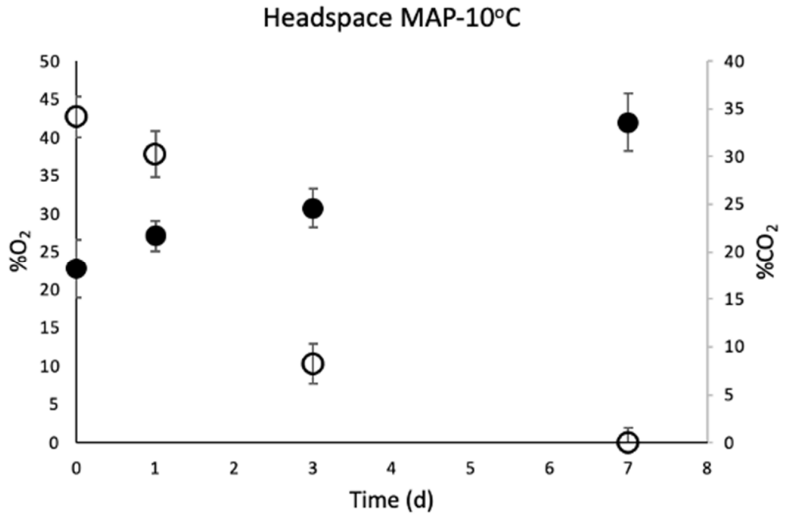


(**c**)

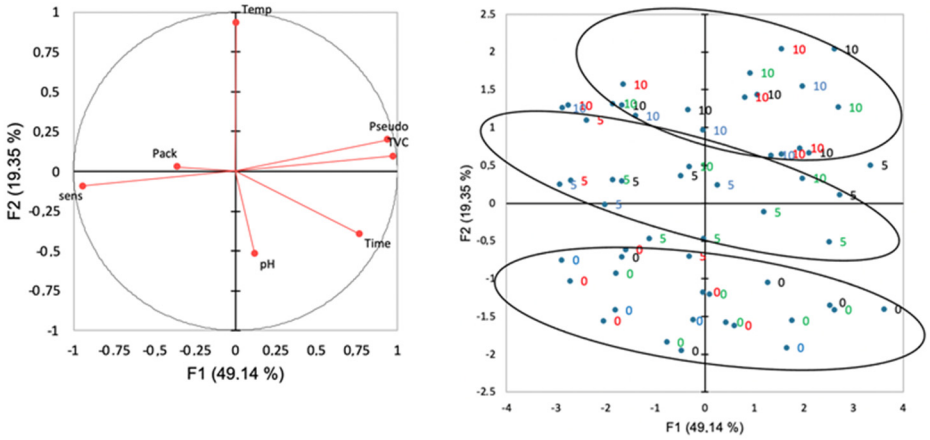
Figure 4. Concentration (%) of ○ O_2 and • CO_2 in the headspace of MAP chicken stored at (**a**) 0 °C, (**b**) 5 °C, and (**c**) 10 °C.

3.5. pH Measurement in Chicken Thighs

The pH of the samples was also measured, but no significant differences ($p > 0.05$) were noted for all samples regardless of the storage temperature, in comparison to the control samples. pH was initially 6.02 ± 0.2 and increased during the first days of storage in all samples stored at 0–10 °C. Similar initial pH values have been reported for fresh chicken cuts [20,21]. At the end of the experiment, pH averaged 6.2 ± 0.2 at 0 °C, 6.1 ± 0.3 at 5 °C, and 6.0 ± 0.3 at 10 °C. Perishable food products packed under vacuum or MAP, for example, poultry, exhibit an increase in pH values due to the growth of spoilage bacteria and the production of basic metabolic compounds [47,48]. In this case, the pH increase may be attributed to the growth of *Pseudomonas* spp., which dominated the spoilage microflora of the chicken samples. These bacteria are known to increase the pH of food due to their metabolic products [54]. The increase in pH of chicken flesh during refrigerated storage may be also attributed to the formation of basic metabolic products of meat spoilage bacteria, for example, ammonia, trimethylamine, and other biogenic amines [20,55].

3.6. Principal Component Analysis (PCA)

In the present study, multivariate statistics (PCA) were used in order to describe the effect of preservation conditions (packaging and temperature) on the quality of fresh chicken cuts during refrigerated storage in the range of 0–10 °C. In the present study, the variability of the obtained data was explained by two components: component 1 explaining 49.35% and component 2 explaining 19.35%. Figure 5a illustrates an inverse relationship between microbial load (i.e., TVC and *Pseudomonas* spp.) and sensory scoring for overall impression, indicating that the higher the microbial load in chicken samples, the lower the sensory scoring that was reported by the panelists. Among the variables, temperature exhibited a higher correlation with component 2 ($R^2 = 0.934$). TVC and *Pseudomonas* spp. load correlated strongly with component 1 ($R^2 = 0.971$ and 0.936, respectively), while for sensory scoring, the respective correlation with component 1 was described by the value $R^2 = 0.945$. Figure 5b illustrates the observations plot of the PCA analysis and the clusters formed as a function of temperature (0, 5 and 10°), indicating that storage temperature plays an important role in the quality of fresh chicken cuts as storage temperature forms, as also indicated by the relatively high E_a values presented in Table 2.



(**a**) (**b**)

Figure 5. Principal component analysis output indicating the effect of packaging and storage conditions on the quality of fresh chicken cuts. (**a**) Variable plot: "Pack" refers to packaging type, "Temp" refers to storage temperature, "TVC" refers to total viable count load, "Pseudo" refers to *Pseudomonas* spp. load, "pH" refers to pH value, and "sens" refers to sensory scoring (overall impression). (**b**) Observation plot: Black refers to the control, green refers to MAP, blue refers to EC-flav, red refers to EC-glu, and numbers 0, 5, and 10 refer to storage temperature, i.e., 0, 5, and 10 °C, respectively.

3.7. Shelf Life Evaluation of Chicken Thighs

Based on the evaluation of various quality parameters during the storage of chicken at different temperatures, the shelf life was estimated for each type of packaging condition. Sensory rejection (scoring 5 for overall impression) of chicken coincided with a TVC level of 7 logCFU/g for all tested storage conditions [47,48,56]. It has been reported in the literature that chicken meat stored at 4 °C for longer than 7 days has been characterised by negative sensory attributes and total viable counts higher than 8 log CFU/g [5].

Shelf life based on the sensory evaluation of chicken was calculated based on Equation (2):

$$t_{SL} = \frac{s_o - s_l}{k_{ref,sens} exp\left[-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]} \quad (2)$$

where t_{SL} is the shelf life of chicken (in d), s_o and s_l are the sensory scoring initially and the rejection time (s_l = 5), $k_{ref\text{-}sens}$ is the sensory scoring reduction rate at the reference temperature T_{ref} = 4 °C, E_a is the activation energy (J/mol), and R is the universal gas constant.

Shelf life based on microbial load was calculated by using Equation (3):

$$t_{SL} = \frac{s_o - s_l}{k_{ref} exp\left[-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]} \quad (3)$$

where t_{SL} is the shelf life of chicken (in d), N_o and N_l are the TVC load initially and at the rejection time (N_l = 7 for TVC), k_{ref} is the microbial growth rate at the reference temperature T_{ref} = 4 °C, E_a is the activation energy (J/mol), and R is the universal gas constant.

Based on the abovementioned calculations, the shelf life of untreated (control) chicken at 0 °C was 8 days based on both Equations (2) and (3), i.e., by using the sensory and microbiological-based criteria. MAP samples exhibited a shelf life of 15 days at 0 °C. EC-flav chicken stored at 0 °C had a shelf life of 19 days by both sensory and microbiological approaches, while the EC-glu samples had a shelf life of 12 days at 0 °C. No significant differences ($p < 0.05$) were observed between the shelf life values obtained based on the sensory scoring and the microbial growth, i.e., Equations (2) and (3). Under this context, the shelf life of chicken at 5 °C was 6–7 days for the control, 11 days for MAP, 13 days for EC-flav, and 7 days for EC-glu samples, respectively. Finally, at 10 °C, the respective shelf life values were 2, 2, 3 and 2 days, indicating that storage temperature plays a crucial role in the preservation of perishable food, such as raw chicken cuts. The obtained shelf life values for refrigerated chicken cuts packed under MA or using edible coatings were in agreement with Giatrakou et al. [44], Patsias at al. [12], and Jiang et al. [57]. Balamatsia et al. [56] reported a strong correlation between the results of the microbiological and sensory analysis, as in the present study. Chicken breast samples stored at 4 °C reached a value of 7 logcfu/g and the score of acceptability of the sensory parameters, after 4–5 days for the aerobically stored samples and after 8 days for the MA packed samples, which was in agreement with the results of the present study, as far as the effect of MAP is concerned. The shelf life of the chicken cuts in the present work was longer as compared to the respective shelf life of chicken samples in the abovementioned study [56]. Finally, the results revealed that TVCs showed a higher relationship to the sensory deterioration of the packaged chicken cuts and, therefore, could be used as an important indicator for the quality of packaged chicken meat freshness and shelf life, which was also shown by other scientists [50].

By using the developed predictive models (Equations (2) and (3)), the shelf life of chicken cuts under the tested packaging methods can be calculated for any storage temperature within the range of 0–10 °C (Figure 6), enabling the prediction of the shelf life of chicken thighs under predetermined storage conditions for the selection of an appropriate packaging system for the desired shelf life of the target food product.

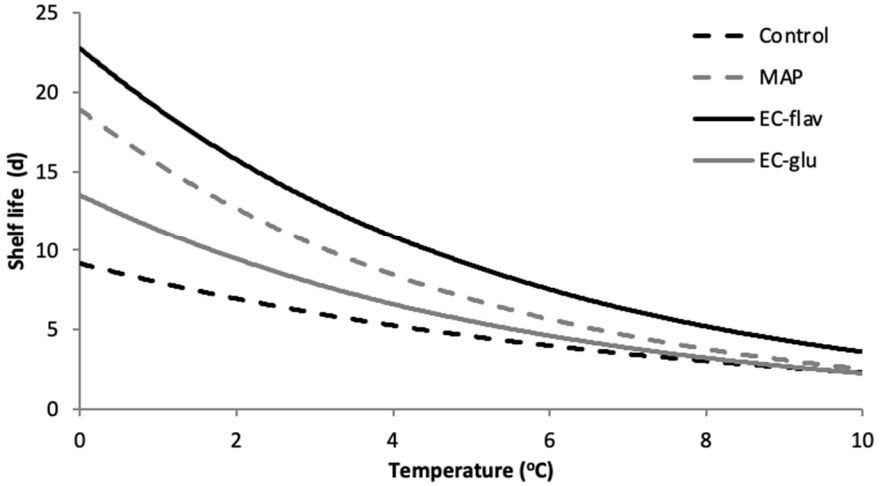


Figure 6. Shelf life (d) of chicken stored at 0–10 °C.

4. Conclusions

The results of the study show the potential of using MAP and edible films with antimicrobial agents to extend the shelf life and improve the commercial value of broiler chicken cuts. The use of edible coatings enriched with glucono-δ-lactone led to a 2-day extended shelf life of chicken samples stored at 5 °C. MAP and active coatings with citrus extract showed a similar effect on the shelf life of chicken samples. The results of the microbiological analysis showed that the shelf life for the control, glucono-δ-lactone coating, MAP, and citrus extract-coated samples were 6, 8, 9, and 9 days, respectively, at 5 °C, revealing an important antimicrobial effect of the edible coatings used in the present study. The use of active packaging applied to the chicken cuts led to the extension of the shelf life and the maintenance of the quality of the chicken meat; therefore, it could be used as an alternative to the conventional packaging commonly used for poultry meat. The proposed active packaging systems may enable a reduction in the use of the currently applied conventional plastic materials in the meat and poultry industry, without limiting product shelf life, thus reducing both food and packaging waste. Further studies are required to validate the developed shelf life predictive models under realistic and dynamic temperature conditions of the real cold chain of perishable food products, such as meat and poultry.

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Conflicts of Interest: The authors declare no conflict of interest.

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