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ORIGINAL ARTICLE

# Shelf-life estimation of sliced Visking-type cooked ham using an active PA-6/chitosan/zeolite-$Ag^+$ packaging

Estimación de la vida útil del jamón Visking rebanado mediante un envase activo PA-6/quitosana/zeolita-$Ag^+$

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**Abstract** The effect of an active film of polyamide 6/chitosan/silver-exchanged zeolite (PA-6/Q/Zeolite-$Ag^+$) on the stability and shelf life of sliced Visking-type cooked ham during refrigerated storage was evaluated, using polyamide 6 (PA-6) bags as a control treatment. Physical, chemical, microbiological, and sensory indicators were analyzed over 60 days of storage, and shelf life was estimated using the Weibull distribution, considering sensory failure as the rejection criterion. The active system reduced the rate of product deterioration, evidenced by greater moisture retention, less acidification, less malondialdehyde accumulation, better preservation of texture, and less growth of aerobic microorganisms and *Pseudomonas* spp. It also delayed the loss of characteristic sensory attributes and the decline in overall quality. Probabilistic analysis determined a sensory shelf life of 38.4 days for PA-6 and 52.19 days for PA-6/Q/Zeolite-$Ag^+$, representing a 35.9% increase. The active film showed potential to extend the stability of sliced cooked ham under refrigeration, primarily by delaying sensory deterioration associated with physicochemical and microbiological changes.

**Keywords** active packaging, sliced cooked ham, chitosan, silver-exchanged zeolite, shelf life, Weibull distribution.

**Resumen** Se evaluó el efecto de una película activa de poliamida 6/quitosana/zeolita intercambiada con plata (PA-6/Q/Zeolita-$Ag^+$) sobre la estabilidad y vida útil de jamón cocido tipo Visking rebanado durante almacenamiento refrigerado, utilizando bolsas de poliamida 6 (PA-6) como tratamiento control. Se analizaron indicadores físicos, químicos, microbiológicos y sensoriales durante 60 días de conservación, y la durabilidad se estimó mediante la distribución de Weibull considerando el fallo sensorial como criterio de rechazo. El sistema activo redujo la velocidad de deterioro del producto, evidenciada por una mayor retención de humedad, menor acidificación, menor acumulación de malondialdehído, mejor conservación de la textura y menor desarrollo de microorganismos aerobios y *Pseudomonas* spp. Asimismo, retrasó la pérdida de atributos sensoriales característicos y la disminución de la calidad global. El análisis probabilístico determinó una vida útil sensorial de 38,4 días para PA-6 y de 52,19 días para PA-6/Q/Zeolita-$Ag^+$, lo que representa un incremento del 35,9 %. La película activa mostró potencial para prolongar la estabilidad del jamón cocido rebanado bajo refrigeración, principalmente mediante el retardo del deterioro sensorial asociado con cambios fisicoquímicos y microbiológicos.

**Palabras clave** envase activo, jamón cocido rebanado, quitosana, zeolita intercambiada con plata, vida útil, distribución de Weibull.

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PUBLIS EDITORIAL

## Introduction

The preservation of refrigerated meat products, particularly ready-to-eat products such as sliced cooked ham, represents a technological challenge because of their high susceptibility to microbiological spoilage and lipid oxidation. These processes limit shelf life and affect sensory acceptability, which has encouraged the development of packaging solutions that go beyond a passive barrier function. In this context, active packaging has gained relevance because of its capacity to interact with the food or its environment, thereby delaying spoilage mechanisms (Yemenicioğlu, 2024).

Innovation in food-packaging materials has explored several strategies, including the incorporation of antimicrobial and antioxidant agents into polymer matrices. Among these, composite systems integrating polyamide, chitosan, and silver-exchanged zeolites have shown potential for applications in meat products (Patiño et al., 2014; Patiño et al., 2022). The combination of a synthetic matrix with structural and barrier functionality, together with active components such as chitosan—because of its antimicrobial properties—and silver zeolite—as a reservoir of metal ions with inhibitory activity—offers a promising approach to extending food stability. However, the effectiveness of these materials cannot be inferred exclusively from in vitro assays and must be validated under actual storage conditions using the specific food product (Dıblan & Kaya, 2023).

Sliced cooked ham is particularly relevant for this evaluation because slicing increases the exposed surface area and the opportunities for contamination after heat treatment. During refrigerated storage, changes in the microbiota—with a predominance of lactic acid bacteria—and their relationship with sensory changes such as increased acidity and the appearance of off-odors and discoloration have been documented (Spampinato et al., 2022). Therefore, shelf-life estimation requires an integrated approach that simultaneously considers physical, chemical, microbiological, and sensory indicators, since loss of acceptability may precede the exceedance of conventional microbiological limits.

To translate this information into a probabilistic shelf-life criterion, Weibull analysis makes it possible to model the temporal distribution of deterioration from units classified as failed or non-failed. This approach, proposed by Núñez de Villavicencio et al. (2017), estimates the scale parameter (characteristic life) and the shape parameter of the distribution and allows determination of the time corresponding to a predefined percentage of deteriorated units, providing a conservative basis for establishing shelf life.

In this context, the objective of this study was to estimate the shelf life of sliced Visking-type cooked ham packaged in bags made from an active polyamide 6/chitosan/silver-exchanged zeolite film, in comparison with conventional polyamide 6 bags, by evaluating its physical, chemical, microbiological, and sensory stability during refrigerated storage (2-5 °C) and applying the Weibull distribution.

## Methodology

A refrigerated-storage trial was conducted to evaluate the effect of an active packaging system on the stability and shelf life of sliced Visking-type cooked ham. Two treatments were compared: a control treatment consisting of ham packaged in polyamide 6 (PA-6) bags and an active treatment in which the product was packaged in bags made from a PA-6/chitosan/silver-exchanged zeolite film (PA-6/Q/Zeolite-$Ag^+$), previously selected through an optimization process for a formulation composed of 96.0% PA-6, 4.0% chitosan, and 5% Ag-zeolite. Through this trial, the physical, chemical, microbiological, and sensory evolution of the product was determined in order to estimate its shelf life during refrigerated storage between 4 and 5 °C.

The experimental follow-up extended to 60 days, according to the final execution of the trial. Physical and chemical determinations were performed at 0, 7, 14, 21, 28, 35, 40, 50, 55, and 60 days of storage, whereas microbiological and sensory evaluations were carried out at 0, 14, 28, 40, 55, and 60 days, covering a time range in which sensory deterioration occurred in both treatments and thus providing sufficient observations for probabilistic shelf-life estimation.

The Visking-type cooked ham used as the food model was obtained from a certified processing plant immediately after production. The product was transported to the laboratory under refrigeration and, under hygienic conditions, sliced into approximately 2-mm-thick slices. Before packaging, the portions were kept in closed containers under refrigeration until use in order to preserve product freshness and obtain a model representative of the commercial conditions of sliced ham.

For the active treatment, films corresponding to the optimized PA-6/chitosan/silver-exchanged zeolite formulation were used. The films were produced by casting, cut into 15 × 15 cm pieces, and paired by heat sealing. The side edges and bottom edge were sealed at 130 °C for 5 s under constant pressure; the sliced ham was then introduced and the top edge was sealed under the same conditions. The control treatment was maintained in PA-6 bags and subjected to the same handling and storage conditions as the active treatment.

For shelf-life evaluation, the packaged samples were maintained under refrigeration at 4 to 5 °C for 60 days. Storage was designed with successive samplings that allowed the temporal evolution of the main deterioration indicators to be characterized using a partially staggered design, with the same number of units evaluated in each period and a reduction in the evaluation interval once the first sensory rejection was observed, thereby concentrating sampling within the experimental period in which the samples were exceeding the practical acceptability limit.

Units intended for physical, chemical, and microbiological analyses were removed at the corresponding times and processed immediately. Portions intended for sensory evaluation were presented to the panel at each established period.

The physical and chemical stability of the ham was evaluated by determining moisture (mass percentage) according to AOAC 950.46 (AOAC International, 2019a), water activity ($a_w$) according to the Krispien method (Rödel et al., 1979), pH (Metrohm Herisau, Model E-510) after homogenizing a 10-g portion in 90 mL of distilled water for 1 min (ISO 2917, 1999), and titratable acidity according to AOAC 942.15 (AOAC International, 2019b), expressed as mass percentage of lactic acid. In addition, lipid oxidation was determined through the concentration of malondialdehyde (MDA), expressed as µmol/kg (García et al., 2016).

For texture analysis, shear force was determined in kilograms (García et al., 2008) using a five-blade Kramer cell (Model HDP/KS5). Samples at 25 °C (20 mm diameter × 10 mm height) were compressed and sheared using a 20-kg load cell at a speed of 100 mm/min in a texturometer (TA-XT2i, Stable Micro Systems Ltd., Godalming) with Texture Expert software.

These were the variables recorded during storage in both packaging systems. The selection of these indicators made it possible to characterize different mechanisms associated with product quality loss.

The microbiological quality of the ham was evaluated at the beginning and after 14, 28, 40, 55, and 60 days of storage. Aerobic microorganisms at 30 °C (ISO 4833-1, 2013), molds and yeasts (ISO 21527-1, 2008), total coliform microorganisms (ISO 4832, 2006), *Escherichia coli* (ISO 7251:2005), *Staphylococcus aureus* (ISO 6888-1, 2021), lactic acid bacteria (LAB) (ISO 15214, 1998), *Pseudomonas* spp. (ISO 13720, 2010), and *Salmonella* spp. (ISO 6579-1, 2017) were determined. Microbial counts were expressed as log CFU/g; coliform microorganisms were reported using the most probable number (MPN), and *Salmonella* spp. as presence/absence.

For interpretation of microbiological stability, the limit of 6 log CFU/g for aerobic mesophilic microorganisms was maintained as a reference. This value was used as a complementary indicator of deterioration and not as the primary event for construction of the probabilistic Weibull model. Likewise, microbiological evolution was used to verify that the loss of sensory acceptability was not accompanied by microbiological conditions incompatible with product stability.

Sensory evaluation was performed by quantitative descriptive analysis (De Pilli et al., 2024). Descriptor generation and reduction were carried out using the controlled association method (Damásio & Costell, 1991), following the criteria of NC-ISO 11035 (2015), including the elimination of semantic redundancies, hedonic terms, attributes with low discriminatory capacity, and the grouping of terms into sensory families. As a result, eight descriptors were selected: pink color for appearance; typical odor and atypical odor for the olfactory dimension; typical flavor, acidic flavor, and rancid flavor for taste; and firmness and juiciness for texture.

The panel consisted of 15 judges. Evaluations were performed according to a balanced complete block design (ISO 13299, 2016), meaning that each judge individually evaluated a portion or slice of ham corresponding to each treatment and storage time and scored the selected descriptors using a structured 10-cm scale with increasing intensity from left to right. Additionally, each judge assigned an overall quality score on a structured 0-to-10-point scale, conceived as the individual integration of the judge's perception of the general condition of the sample.

Sensory evaluations were performed at 0, 14, 28, 40, 55, and 60 days. Overall quality was used exclusively to define the acceptance or rejection condition subsequently used in the shelf-life analysis. The eight descriptors did not constitute, separately, mandatory failure criteria, but rather explanatory variables for the judge's overall decision. Consequently, no specific combination of defects was established beforehand as necessary to declare rejection of a sample. Each judge integrated the different attributes sensorially and assigned an overall quality score.

The judges could supplement each evaluation with qualitative observations intended to support the overall score assigned. These comments, together with the QDA descriptor scores, were used to interpret the sensory characteristics associated with loss of acceptability, but they did not modify the quantitative criterion used to classify failure.

For the probabilistic shelf-life analysis, a cutoff point of 5 was established a priori for overall quality (OQ). An individual response was classified as non-failure or acceptable product when overall quality was equal to or greater than 5 points ($OQ \geq 5$), whereas failure or sensory rejection was considered when the score was below 5 points ($OQ < 5$).

The individual response of each judge, obtained from the evaluation of a portion or slice at a given time, constituted the observation unit for failure analysis. Thus, each evaluation time provided 15 individual observations per treatment. Considering the six sensory evaluation times, the design generated up to 90 acceptance/rejection observations for each packaging system. A time was considered a failure time when a minimum significant number of judges agreed, based on a binomial distribution with $p = 0.01$. The use of the individual overall quality score made it possible to operationalize the failure event explicitly and reproducibly before probabilistic fitting.

The sensory shelf life of the ham for each packaging system was estimated using the direct Weibull analysis method or hazard technique described by Núñez de Villavicencio et al. (2017). This procedure is applicable to refrigerated foods with short or medium shelf life and uses the life times and failure/non-failure condition of the evaluated units. Once the storage and characterization trials were completed, the life times of the experimental units could be used to estimate product shelf life probabilistically.

The analysis was performed independently for the control treatment and the active treatment. Observations were ordered in ascending order according to storage time, while retaining the failure or non-failure classification for each record. An inverse rank (*k*) was then assigned to each observation, calculated as:

$$k = n - i + 1 \quad \text{Equation 1}$$

where n represents the number of observations included in the analysis and i the position of each observation in the ordered series. For each observation classified as a failure, the instantaneous hazard (h) was calculated using Equation 2:

$$h(t) = \frac{1}{k} \quad \text{Equation 2}$$

The cumulative hazard *H(t)* was obtained by sequentially summing the hazard corresponding to each failure and the hazards associated with preceding failures. This procedure reproduces the hazard plotting technique (Núñez de Villavicencio et al., 2017), in which non-failed observations remain within the set exposed to risk but do not add a hazard increment.

The parameters of the Weibull distribution were obtained from the linearized relationship between storage time and cumulative hazard according to Equation 3:

$$\ln(t) = \ln(\alpha) + \frac{1}{\beta} ln[H(t)] \quad \text{Equation 3}$$

where α corresponds to the scale parameter or characteristic life and β to the shape parameter. The model intercept allowed estimation of ln(α), whereas the slope corresponded to 1/β. According to the procedure of Núñez de Villavicencio et al. (2017), observations whose cumulative hazard was greater than 1 were excluded from the linear fit. The Weibull cumulative distribution function was expressed as:

$$F(t) = 1 - exp\left[-\left(\frac{t}{\alpha}\right)^{\beta}\right] \quad \text{Equation 4}$$

where *F(t)* represents the cumulative probability that a unit has experienced sensory failure by time t. Complementarily, the probability of remaining in an acceptable condition was expressed through the following survival function:

$$S(t) = 1 - F(t) = exp\left[-\left(\frac{t}{\alpha}\right)^{\beta}\right] \quad \text{Equation 5}$$

The parameters α and β were estimated together with their 95% confidence intervals. The adequacy of the Weibull distribution to the failure data was verified using the modified Kolmogorov-Smirnov test, following the procedure indicated by Núñez de Villavicencio et al. (2017). The model was considered appropriate when, at a 95% confidence level, the hypothesis that the failure times came from a Weibull distribution could not be rejected.

Once the parameters α and β had been estimated, the times corresponding to different percentiles of the distribution were calculated as follows:

$$t_p = \alpha[-\ln(1-p)]^{1/\beta} \quad \text{Equation 6}$$

where p represents the admitted cumulative proportion of deteriorated units. Núñez de Villavicencio et al. (2017) defined these percentiles as the percentage of deteriorated units accepted within a lot and proposed their use for selecting product shelf life.

In the present study, a maximum of 5% of units with sensory failure was established as the primary shelf-life criterion. Therefore, probabilistic shelf life corresponded to the 5th percentile of the Weibull distribution ($B_5$ or $t_{0.05}$):

$$t_{0.05} = \alpha[-\ln(0{,}95)]^{1/\beta} \quad \text{Equation 7}$$

This criterion implies an estimated probability of 95% that a unit remains sensorially acceptable up to the time established as shelf life. The selection of 5% is consistent with the procedure developed by Núñez de Villavicencio et al. (2017).

In addition to $B_5$, the $B_1$, $B_{10}$, $B_{20}$, $B_{25}$, $B_{50}$, $B_{75}$, $B_{90}$, $B_{95}$, and $B_{99}$ percentiles were calculated, as appropriate, to comprehensively characterize the temporal distribution of deterioration risk. $B_5$ was nevertheless retained as the main shelf-life estimator. Values were accompanied by 95% confidence intervals. When shelf life was expressed in whole days for technological purposes, the continuous $B_5$ value was conservatively rounded down to the next lower integer, following the criterion of Núñez de Villavicencio et al. (2017), who established this approximation for the selected percentile.

Physical and chemical results were analyzed considering packaging system and storage time as experimental factors. A two-way analysis of variance (ANOVA) (treatment

× time) was applied to determine the main effects and their interaction. When significant differences were identified, means were compared using Tukey's HSD multiple-comparison test at a significance level of $p \leq 0.05$. Model fit quality was verified through residual analysis and the corresponding coefficients of determination. STATISTICA software (v. 7, 2005, StatSoft Inc., Tulsa) was used for statistical processing.

Scores for the QDA descriptors and overall quality were analyzed as a function of treatment and storage time. For sensory attributes, the judge was considered a blocking factor because individual scores were obtained from the same panel throughout the evaluation sessions. Descriptive information from the eight attributes was used to interpret changes in the sensory profile and their relationship with the decline in overall quality. A two-way ANOVA (time × treatment) was applied, together with integration of the sensory information with microbiological and oxidation indicators.

Weibull analysis was performed independently for each treatment using individual failure/non-failure data. For each distribution, α, β, their 95% confidence intervals, cumulative failure probability *F(t)*, survival function *S(t)*, and life-time percentiles were estimated. Comparison between packaging systems was based primarily on the displacement of the failure curves and differences between their $B_5$ estimates, using the corresponding confidence intervals to express the uncertainty associated with the estimate.

## Results and discussion

The physical and chemical properties of Visking-type cooked ham changed during the 60 days of storage in both packaging systems. The samples showed similar initial conditions; subsequently, moisture, water activity, pH, and shear force decreased, whereas acidity and MDA increased. Overall, these changes were less pronounced in the PA-6/chitosan/zeolite-$Ag^+$ treatment.

Moisture decreased from 72.0 to 62.5% in PA-6 and to 65.5% in the active material (Figure 1), corresponding to losses of 9.5 and 6.5 percentage points, respectively. Differences between treatments were observed from day 28 onward, indicating greater water retention in ham packaged with PA-6/chitosan/zeolite-$Ag^+$. Similar results were reported by Chang et al. (2019), who associated improved barrier properties of chitosan materials with lower drip losses in refrigerated meat. Although the system used by those authors differs from the material evaluated here, both findings show that incorporating chitosan into packaging structures can help reduce water loss when barrier properties are favorably modified.

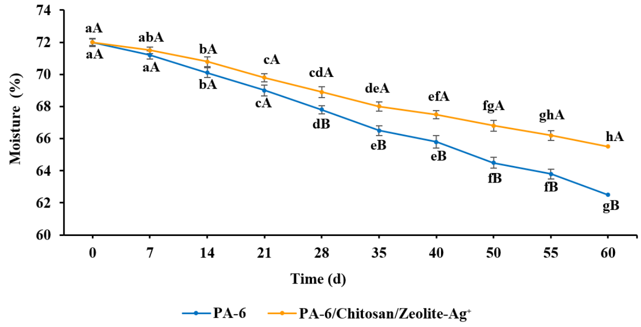


**Figure 1.** Changes in moisture content of Visking-type cooked ham during refrigerated storage in the two packaging systems. Different lowercase letters indicate significant differences among storage times within the same packaging system; different uppercase letters indicate significant differences between packaging systems at the same storage time according to Tukey HSD multiple comparisons, $p \leq 0.05$.

Water activity ($a_w$) decreased from 0.975 to 0.955 in PA-6 and to 0.957 in the active material (Figure 2). No differences were observed between packaging systems; therefore, the greater moisture retention in the active treatment was not accompanied by an appreciable change in water availability. This behavior agrees with Patiño et al. (2022), who also found no differences in $a_w$ in sausages packaged in a PA-6/silver-zinc system. Therefore, moisture content and a_w should be interpreted independently because they represent different properties of the water present in the food.

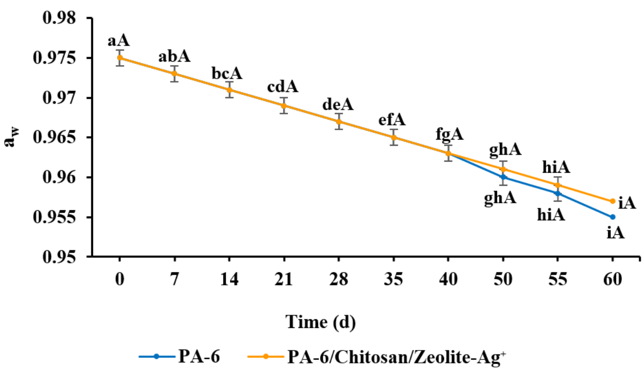


**Figure 2.** Changes in water activity of Visking-type cooked ham during refrigerated storage in the two packaging systems. Different lowercase letters indicate significant differences among storage times within the same packaging system; different uppercase letters indicate significant differences between packaging systems at the same storage time according to Tukey HSD multiple comparisons, $p \leq 0.05$.

The pH decreased in both treatments (Figure 3), whereas acidity increased during storage (Figure 4). In PA-6, pH decreased from 6.40 to 5.45 and acidity increased from 0.86 to

1.55%. In the active material, the final values were 5.70 and 1.40%, respectively, indicating less intense acidification.

The smaller change in pH in the active treatment differs from the findings of Patiño et al. (2022), who observed no effect of a PA-6/silver-zinc system on the pH of beef and chicken sausages. These differences may be related to material composition, product type, and storage time, confirming the need to evaluate each active system in the food matrix of interest.

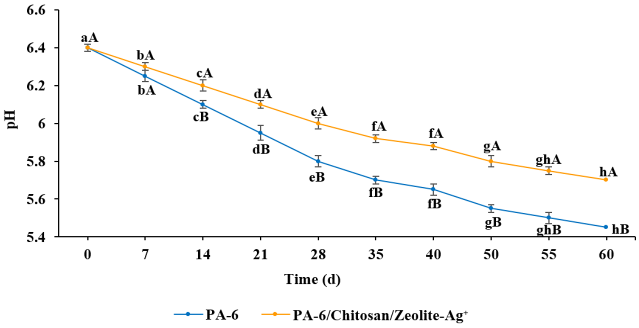


**Figure 3.** Changes in pH of Visking-type cooked ham during refrigerated storage in the two packaging systems. Different lowercase letters indicate significant differences among storage times within the same packaging system; different uppercase letters indicate significant differences between packaging systems at the same storage time according to Tukey HSD multiple comparisons, $p \leq 0.05$.

Figure 4 shows the increase in acidity during storage. From day 28 onward, differences between treatments became more evident. The PA-6 treatment showed 1.15 ± 0.03%, compared with 1.08 ± 0.02% in the active material, and on day 60 the values were 1.55 ± 0.03 and 1.40 ± 0.02%, respectively. The effects of treatment, time, and their interaction were significant ($p \leq 0.05$).

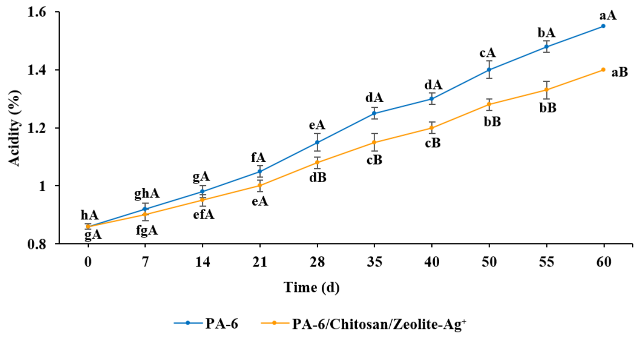


**Figure 4.** Changes in acidity of Visking-type cooked ham during refrigerated storage in the two packaging systems. Different lowercase letters indicate significant differences among storage times within the same packaging system; different uppercase letters indicate significant differences between packaging systems at the same storage time according to Tukey HSD multiple comparisons, $p \leq 0.05$.

Spampinato et al. (2022) observed accumulation of lactic and acetic acids during storage of sliced cooked ham, accompanied by acidic taste and spoilage odors. The lower acidification observed with the active material was consistent with slower development of these changes. However, in the present study, LAB did not proliferate but decreased in both treatments. Therefore, the decrease in pH and increase in acidity could not be attributed solely to this microbial group and may be related to the combined action of chemical and microbiological processes.

Shear force decreased from 25.0 to 17.2 kg in PA-6 and to 19.2 kg in the active material (Figure 5), representing reductions of 31.2 and 23.2%, respectively. The differences observed at the later storage times show that the active system delayed changes in product texture. These results partially differ from those of Patiño et al. (2022), who found no effect of PA-6/silver-zinc material on sausage texture. In contrast, Chang et al. (2019) associated improved barrier properties of chitosan materials with lower fluid losses in refrigerated meat, which could contribute to maintaining texture. In the present study, the concurrence of greater moisture retention and higher shear force suggests an association between both phenomena, although it does not establish a causal relationship.

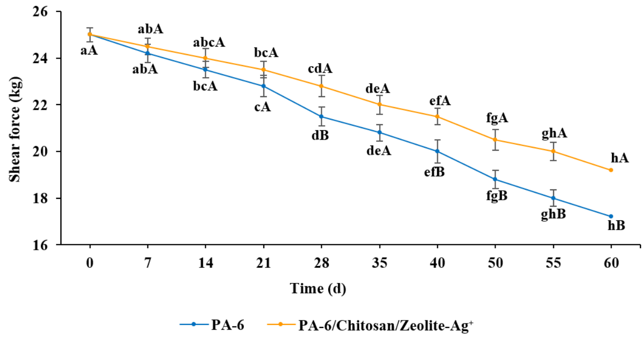


**Figure 5.** Changes in shear force of Visking-type cooked ham during refrigerated storage in the two packaging systems. Different lowercase letters indicate significant differences among storage times within the same packaging system; different uppercase letters indicate significant differences between packaging systems at the same storage time according to Tukey HSD multiple comparisons, $p \leq 0.05$.

Figure 6 shows the increase in MDA during storage. On day 28, values of 1.75 µmol/kg were recorded in PA-6 and 1.05 µmol/kg in the active material; by day 60, the values reached 3.35 and 2.00 µmol/kg, respectively. The final value in the active treatment was approximately 40% lower, indicating a lower progression of lipid oxidation.

The lower MDA content is consistent with findings in other meat products. Patiño et al. (2022) reported lower TBARS values in sausages packaged with PA-6/silver-zinc, whereas Suman et al. (2010) observed reduced lipid oxidation in beef treated with chitosan. Similarly, Ruiz-Navajas et al. (2015) found lower oxidation in cooked cured ham protected with chitosan materials containing essential oils.

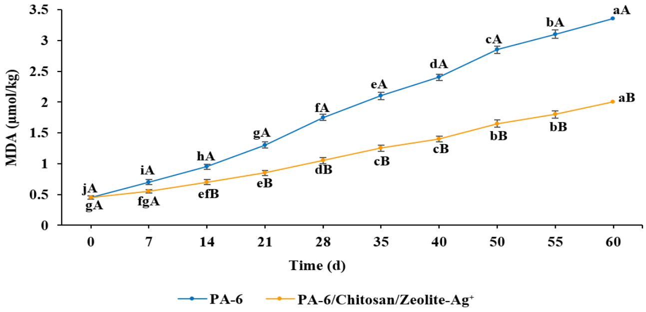


**Figure 6.** Changes in malondialdehyde content of Visking-type cooked ham during refrigerated storage in the two packaging systems. Different lowercase letters indicate significant differences among storage times within the same packaging system; different uppercase letters indicate significant differences between packaging systems at the same storage time according to Tukey HSD multiple comparisons, $p \leq 0.05$.

Absolute MDA concentrations should not be directly compared with TBARS values obtained using other analytical procedures. The most appropriate comparison is based on the relative trend versus the control. In addition, the experimental design does not allow the individual contributions of chitosan, zeolite, and silver to be separated; therefore, the observed effect should be attributed to the composite material as a whole.

Overall, the PA-6/chitosan/zeolite-$Ag^+$ material delayed moisture loss, acidification, changes in shear force, and, more markedly, lipid oxidation, without significantly modifying water activity. This behavior is consistent with the selective response reported for other active systems based on polyamide, chitosan, and silver compounds (Patiño et al., 2022; Dıblan & Kaya, 2023).

Microbiological changes were characterized by low counts throughout the 60 days of storage (Table 1). Aerobic microorganisms at 30 °C increased in both treatments, although the increase was smaller in the active material. Molds, yeasts, coliforms, and *S. aureus* remained below the established limits, whereas *Salmonella* spp. was absent.

Aerobic microorganisms at 30 °C increased from 2.0 to 2.48 log CFU/g in PA-6 and from 1.9 to 2.23 log CFU/g in the active material. This increase was slower in PA-6/chitosan/zeolite-$Ag^+$ and agreed with Patiño et al. (2022), who observed lower development of aerobic microorganisms in sausages packaged with PA-6/silver-zinc.

Molds and yeasts remained at < 1 log CFU/g, *S. aureus* at < 2 log CFU/g, and coliforms at < 3 MPN throughout storage. The absence of differences between treatments for these groups indicates that their low counts mainly reflected the hygienic stability of the product rather than a demonstrable differential effect of the active material.

LAB decreased from 1.54 to 1.0 log CFU/g in PA-6 and from 1.48 to 1.0 log CFU/g in the active material. This behavior contrasts with Kreyenschmidt et al. (2010) and Spampinato et al. (2022), who described LAB as one of the main microbial groups associated with spoilage of cooked ham. The differences may be related to the initial load, formulation, packaging system, and storage conditions.

The greatest microbiological difference was observed for *Pseudomonas* spp. In the PA-6 system, the count increased from 1.4 to 2.04 log CFU/g, whereas in the active material it decreased from 1.3 to 1.0 log CFU/g by day 28 and remained < 1 log CFU/g from day 40 onward.

**Table 1.** Microbiological changes in Visking-type cooked ham during refrigerated storage (4 to 5 °C)

| System | Time (days) | Microorganisms (log CFU/g) | | | | | | | Microorganisms (MPN) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Aerobes at 30 °C | Molds | Yeasts | *S. aureus* | LAB | *Pseudomonas* spp. | *Salmonella* spp. | TC | FC |
| PA-6 | 0 | 2.00 | < 1 | < 1 | < 2 | 1.54 | 1.40 | Absent | < 3 | < 3 |
| | 14 | 2.15 | < 1 | < 1 | < 2 | 1.48 | 1.54 | Absent | < 3 | < 3 |
| | 28 | 2.26 | < 1 | < 1 | < 2 | 1.40 | 1.70 | Absent | < 3 | < 3 |
| | 40 | 2.34 | < 1 | < 1 | < 2 | 1.30 | 1.85 | Absent | < 3 | < 3 |
| | 55 | 2.41 | < 1 | < 1 | < 2 | 1.18 | 1.95 | Absent | < 3 | < 3 |
| | 60 | 2.48 | < 1 | < 1 | < 2 | 1.00 | 2.04 | Absent | < 3 | < 3 |
| PA-6/ Chitosan/ Zeolite-$Ag^+$ | 0 | 1.90 | < 1 | < 1 | < 2 | 1.48 | 1.30 | Absent | < 3 | < 3 |
| | 14 | 1.95 | < 1 | < 1 | < 2 | 1.40 | 1.18 | Absent | < 3 | < 3 |
| | 28 | 2.04 | < 1 | < 1 | < 2 | 1.30 | 1.00 | Absent | < 3 | < 3 |
| | 40 | 2.11 | < 1 | < 1 | < 2 | 1.18 | <1.00 | Absent | < 3 | < 3 |
| | 55 | 2.18 | < 1 | < 1 | < 2 | 1.00 | <1.00 | Absent | < 3 | < 3 |
| | 60 | 2.23 | < 1 | < 1 | < 2 | 1.00 | <1.00 | Absent | < 3 | < 3 |

LAB: lactic acid bacteria; TC: total coliforms; FC: fecal coliforms (*Escherichia coli*).

Darmadji and Izumimoto (1994) reported chitosan activity against *Pseudomonas fragi*, and Rhim et al. (2006) observed antimicrobial activity in chitosan materials containing Ag-zeolite. These findings support the observed response, although the present design does not allow the contribution of each component to be identified separately.

The microbiological indicators did not reach the value adopted as the spoilage reference and therefore did not define the end of shelf life. The effect of the active material was mainly reflected in the behavior of *Pseudomonas* spp. and, to a lesser extent, aerobic microorganisms at 30 °C.

The counts were lower than those reported by Spampinato et al. (2022) during the secondary shelf life of sliced cooked ham, in which aerobic microorganisms and LAB reached high values. This difference does not by itself indicate superiority of the system studied because the storage conditions and microbiological stage of evaluation were different. Ruiz-Navajas et al. (2015) also reported that the microbiota of cooked hams may vary depending on processing and the preservation system.

The sensory profile comprised eight descriptors: pink color, typical odor, atypical odor, typical flavor, acidic flavor, rancid flavor, firmness, and juiciness. Changes in these attributes showed a faster loss of typical characteristics in PA-6 (Figure 7).

Typical odor decreased from 9.1 to 1.0 points in PA-6 and to 5.5 points in the active material, whereas atypical odor increased to 7.5 and 3.5 points, respectively. Differences between treatments became more evident after 28–40 days.

Similar results were reported by Spampinato et al. (2022), who associated spoilage of sliced cooked ham with the development of atypical odors and acidic taste. Serio et al. (2018) also observed that chitosan coatings delayed the appearance of unpleasant odors in refrigerated pork. The greater preservation of typical odor in the present study was consistent with these findings.

Flavor followed a similar trend. In PA-6, typical flavor decreased to 5.0 points on day 40 and 1.0 point on day 60; in the active material, it remained at 7.2 and 4.8 points at the same storage times. Acidic flavor reached 3.0 points in PA-6 and 2.0 points in the active treatment.

Rancid flavor remained absent until day 28 and subsequently increased to 4.0 points in PA-6 and 1.8 points in the active material by day 60. This difference followed the same trend as MDA and suggests an association between the progression of lipid oxidation and the perception of rancidity.

The decrease in typical flavor and increase in acidic flavor agreed with that described by Spampinato et al. (2022) for sliced cooked ham. Similarly, Siripatrawan and Noipha (2012) observed better sensory preservation of pork sausages with active chitosan materials.

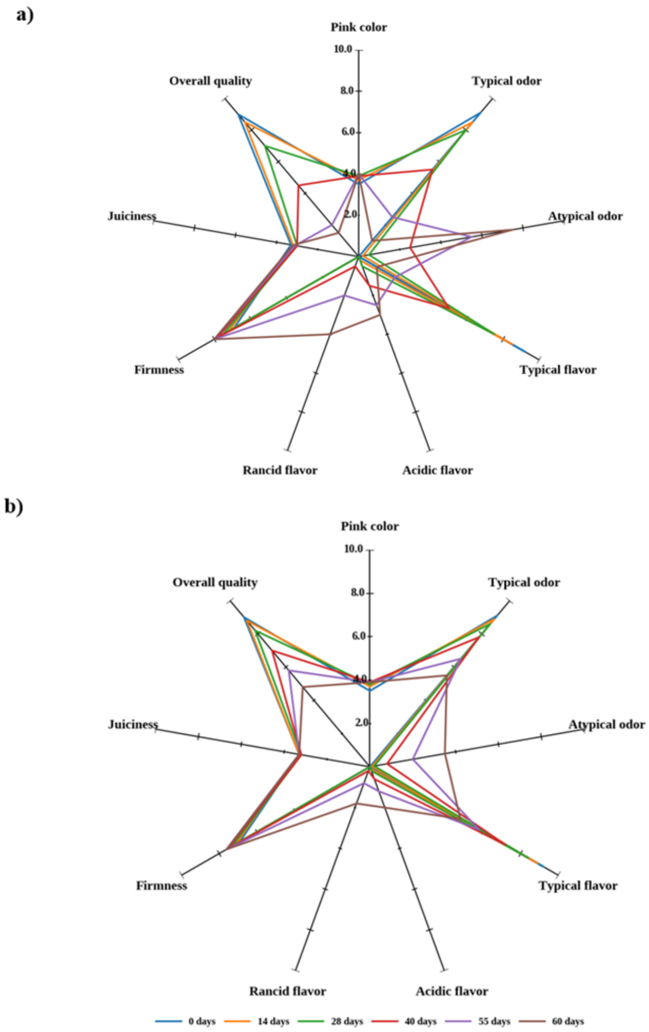


**Figure 7.** Sensory profile of Visking-type cooked ham during refrigerated storage in both packaging systems. a) PA-6. b) PA-6/chitosan/zeolite-$Ag^{+}$.

Firmness and juiciness varied less than odor and flavor attributes. Firmness increased moderately during storage, whereas juiciness remained between 3.0 and 3.3 points, with no relevant differences between systems. Patiño et al. (2022) likewise found no significant differences in the textural attributes of sausages packaged with PA-6/silver-zinc. In cooked ham, Barbieri et al. (2016) indicated that texture and juiciness contribute to differentiating product quality; however, in the present study, these descriptors were not the attributes that best discriminated deterioration between treatments.

Pink color showed minimal changes and no differences were observed between treatments. On day 60, values were 4.0 ± 0.35 points in PA-6 and 3.9 ± 0.35 points in the active material. This behavior agreed with Patiño et al. (2022), who observed no effect of the PA-6/silver-zinc system on the color of chicken and beef sausages.

Overall quality most clearly integrated the progression of deterioration (Figure 8). In PA-6, it decreased from 9.0 to 7.0

points between days 0 and 28 and reached 4.5 points on day 40. In the active material, it remained at 8.2 points on day 28, 7.0 on day 40, and 5.8 on day 55; on day 60 it reached 4.8 points.

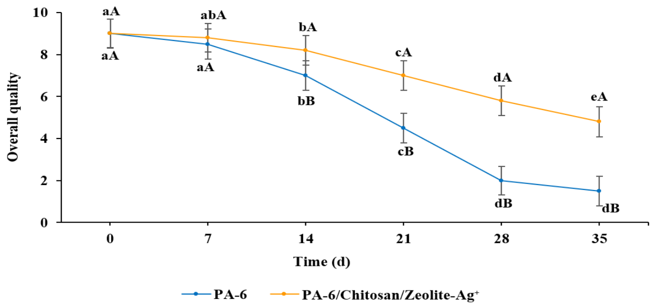


**Figure 8.** Changes in overall quality of Visking-type cooked ham during refrigerated storage in the PA-6 and PA-6/chitosan/zeolite-$Ag^+$ packaging systems. Different lowercase letters indicate significant differences among storage times within the same packaging system; different uppercase letters indicate significant differences between packaging systems at the same storage time according to Tukey HSD multiple comparisons, $p \leq 0.05$.

These results show that the active material delayed the loss of acceptability. The point at which the mean fell below 5 was used only as a descriptive reference because shelf life was estimated from the individual responses of the panelists. Spampinato et al. (2022) observed that loss of acceptability in cooked ham resulted from a combination of acidic taste, unpleasant odors, and appearance changes. Similarly, Siripatrawan and Noipha (2012) reported better maintenance of overall acceptability in sausages protected with active chitosan materials.

Integration of the results showed greater stability of ham packaged with PA-6/chitosan/zeolite-$Ag^+$. From day 40 onward, the PA-6 treatment showed greater moisture loss, acidification, and MDA accumulation, together with poorer preservation of typical odor and flavor and greater intensity of atypical odor, acidic flavor, and rancidity. In the active material, these changes were delayed and occurred with lower intensity.

Because the indicator microorganisms remained below the established limits and *Salmonella* spp. was absent, the results suggested that the end of shelf life was determined mainly by sensory rejection, whereas physicochemical and microbiological changes acted as complementary indicators of deterioration, in agreement with the probabilistic Weibull analysis.

Shelf life was estimated from the individual overall-quality scores assigned by the 15 panelists (Table 2). Responses with $OQ < 5$ were classified as sensory failures and those with $OQ \geq 5$ as acceptable. The distribution of failures showed a shift in rejection toward longer storage times in PA-6/chitosan/zeolite-$Ag^+$. No failures were recorded in the PA-6 treatment until day 28; on day 40, 10 of 15 responses were classified as rejection (66.7%), and from day 55 onward failure reached 100%.

**Table 2.** Changes in overall quality and frequency of sensory failure during refrigerated storage

| Treatment | Time (d) | Overall quality (Mean ± SD) | Failures (OQ < 5) | Failures (%) |
|---|---|---|---|---|
| PA-6 | 0 | 9.0 ± 0.68 | 0/15 | 0.0 |
| | 14 | 8.5 ± 0.71 | 0/15 | 0.0 |
| | 28 | 7.0 ± 0.71 | 0/15 | 0.0 |
| | 40 | 4.5 ± 0.70 | 10/15 | 66.7 |
| | 55 | 2.0 ± 0.68 | 15/15 | 100 |
| | 60 | 1.5 ± 0.70 | 15/15 | 100 |
| PA-6/ Chitosan/ Zeolite-$Ag^+$ | 0 | 9.0 ± 0.70 | 0/15 | 0.0 |
| | 14 | 8.8 ± 0.69 | 0/15 | 0.0 |
| | 28 | 8.2 ± 0.70 | 0/15 | 0.0 |
| | 40 | 7.0 ± 0.71 | 0/15 | 0.0 |
| | 55 | 5.8 ± 0.70 | 2/15 | 13.3 |
| | 60 | 4.8 ± 0.71 | 8/15 | 53.3 |

OQ: overall quality; SD: standard deviation. An individual OQ score < 5 was considered a sensory failure.

No failures were recorded in the active material until day 40; on day 55, 2 of 15 responses were failures (13.3%), and on day 60, 8 of 15 (53.3%).

Individual failure/non-failure responses were fitted to a two-parameter Weibull distribution by maximum likelihood. $B_5$ and $B_{50}$ were estimated from α and β (Table 3). $B_5$ was used as the primary shelf-life criterion, according to the admissible percentage of deteriorated units proposed by Núñez de Villavicencio et al. (2017).

The scale parameter α increased from 39.95 days in PA-6 to 60.84 days in the active material. The β values were 74.86 and 19.37, respectively. In both cases, $\beta > 1$ indicates an increasing risk of failure over time; the higher magnitude in PA-6 reflects a more concentrated transition toward rejection.

**Table 3.** Weibull distribution parameters and sensory shelf-life estimates

| Parameter | PA-6 | PA-6/Chitosan/ Zeolite-$Ag^+$ |
|---|---|---|
| Scale parameter, α (days) | 39.95 | 60.84 |
| Shape parameter, β | 74.86 | 19.37 |
| $B_5$ (days) | 38.40 | 52.19 |
| $B_{50}$ (days) | 39.75 | 59.70 |
| Increase in $B_5$ relative to PA-6 | - | 35.9 % |

$B_5$ was 38.4 days for PA-6 and 52.19 days for PA-6/chitosan/zeolite-$Ag^+$. This represents an extension of 13.79 days and an increase of 35.9%. $B_{50}$ increased from 39.75 to 59.70 days, confirming the shift of the failure distribution toward longer storage times.

The use of Weibull analysis allowed shelf life to be expressed as a probability of failure rather than only as the time at which a sensory mean crosses a limit. Cardelli and Labuza (2001) and Duyvesteyn et al. (2001) used this approach to estimate shelf life from rejection, whereas Calle et al. (2006) and Hough et al. (2006) demonstrated the usefulness of survival analysis for incorporating variability in sensory responses.

The closest precedent is Patiño et al. (2022), who used a PA-6/silver-zinc packaging system for beef and chicken sausages and estimated a $B_5$ of 26.6 days for the control and 35.62 days for the active material at 4 °C, equivalent to an increase of approximately 33.9%. This value is close to the 35.9% increase obtained in the present study.

Although the products and materials differ, both studies show an extension of shelf life of approximately one-third compared with the conventional system. The greater absolute difference observed in ham may be related to material composition and to product-specific deterioration mechanisms, although it cannot be attributed to any single component.

Patiño et al. (2022) also showed that the effect of active packaging depended on the product and storage temperature. This confirms that shelf-life extension is not a fixed property of the material but rather the result of its interaction with the food matrix and storage conditions. The findings of Dıblan and Kaya (2023) support this interpretation. In Kaşar cheese, multilayer materials containing chitosan or silver-substituted zeolite prolonged microbiological stability, although effectiveness depended on the microorganism and temperature.

The difference between $B_5$ and $B_{50}$ was 1.35 days in PA-6 and 7.51 days in the active material. This greater separation, together with the lower β, indicated that rejection of the active treatment was distributed over a wider time interval. $B_{50}$ describes the median failure time, whereas $B_5$ provides a more conservative criterion for establishing shelf life. The use of rejection probabilities to define sensory limits has been recommended in food survival studies (Hough et al., 2004; Garitta et al., 2015).

Overall, the increase in $B_5$ from 38.4 to 52.19 days was consistent with the shelf-life extension reported for other active materials containing polyamide, chitosan, or silver species (Patiño et al., 2022; Dıblan & Kaya, 2023). The shift in α, $B_5$, and $B_{50}$ toward longer times indicates that the PA-6/chitosan/zeolite-$Ag^+$ system delayed sensory rejection of Visking-type cooked ham during refrigerated storage.

## Conclusions

The active PA-6/chitosan/silver-exchanged zeolite film improved the overall stability of sliced Visking-type cooked ham during refrigerated storage compared with conventional PA-6 packaging. The active system delayed moisture loss, acidification, lipid oxidation, and textural changes, while also limiting the growth of aerobic microorganisms and exerting an inhibitory effect on *Pseudomonas* spp. Shelf-life loss was mainly determined by sensory deterioration, associated with a decline in characteristic attributes and an increase in defects such as atypical odor, sour taste, and rancidity, whereas microbiological indicators remained within acceptable limits. The Weibull model estimated a sensory shelf life of 38.4 days for PA-6 and 52.19 days for PA-6/Q/Zeolite-$Ag^+$, corresponding to an extension of approximately 13.8 days, or 35.9%, compared with the conventional system. Therefore, the incorporation of chitosan and silver-exchanged zeolite into polyamide films represents an effective technological strategy for extending the shelf life of refrigerated sliced cooked ham.

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**Conflicts of interest**

The authors declare that they have no conflicts of interest.

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**Data availability statement**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Statement on the use of AI**

The authors acknowledge the use of generative AI and AI-assisted technologies to improve the readability and clarity of the article.

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