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Effect of nanocomposite packaging containing different proportions of ZnO and Ag on chicken breast meat quality

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Section: Processing, Products and Food Safety.

Highlights

We studied the antimicrobial power of a combination of ZnO and Ag in LDPE packaging

We studied the influence of this packaging on chicken breast shelf life and quality.

ZnO+Ag nanoparticles had an antimicrobial effect and delayed lipid oxidation

ZnO+Ag nanoparticles migrate into the food in amounts below those allowed by law.

ABSTRACT

This study reports the antimicrobial capacity, nanoparticle migration properties and the influence on some meat quality traits of a packaging based on a low density polyethylene (LDPE) blended with a nano-antimicrobial master batch composed of Ag and ZnO (5 % and 10% w/w). Meat was aged for storage times of 0, 7, 10, 15 or 21 days. Composition of the package atmosphere, some microbiological analyses, meat sensorial quality, meat color, visual appearance score and lipid oxidation index (TBAR) values were determined. Irrespective of the packaging, the O_2 concentration decreased and the CO_2 concentration and count for all types of microbe increased with storage time. Redness, yellowness and Chroma of the breast meat increased until to 7 days whereas maximum lightness values were found at 15 days. Visual appearance scores decreased and lipid oxidation increased with storage time. It was found that adding ZnO+Ag nanoparticles to LDPE packaging has an antimicrobial effect whilst migration amounts were well within those allowed by law.

Key words: active packaging, meat quality**,** microbiology, migration, nanoparticle

INTRODUCTION

The appropriate packaging of poultry meat products is vital if food safety and product shelf-life are to be guaranteed. In recent years, interest has been growing in the use of intelligent or active packaging that can meet producers' and consumers' demands for products with longer shelf lives (Kerry *et al.*, 2006). One possible solution is the incorporation of nanoparticles into composite packaging materials to enhance the mechanical and chemical properties of the polymer base material.

'Nano food packaging' with antimicrobial properties represents a new generation of active packaging based on metal nanocomposites. Inorganic materials such as metals and metal oxides have been the focus of nanotechnology research.

Despite the variety of antimicrobial agents, the search for effective biocidal agents has focused on the development of nanostructures of certain metals such as silver, copper, zinc and gold (Sondi, 2004). However, given their reduced cost, recent research efforts have focused on the use of ZnO nanoparticles (Tankhiwale and Bajpai,, 2012). ZnO is particularly interesting since it appears to cause no harm to either animals or humans (Lin *et al.*, 2009; Stoimenov *et al.*, 2002) but has a strong antimicrobial effect on a broad spectrum of microorganisms. Silver has also long been known to inhibit microbial growth (Emamifar *et al.*, 2010). There are several ways to incorporate nanoparticles into packaging plastics, but the most commonly used method is melt mixing (Damm *et al.*, 2006).

A disadvantage of using nanoparticles in packaging is the possibility of their migration towards the packaged food, potentially causing toxicity problems. Migration tests must therefore be performed when new nano food packaging is designed. European legislation controls the compounds that can be used in the manufacture of containers intended to hold food, as well as the conditions under which migration studies must be performed (EU Directive 19/2007/EC). A further potential disadvantage is that nanoparticles might affect meat quality, especially its color and the oxidation of fat (which can cause flavor problems).

The aims of the present work were to study the antimicrobial power of a combination of ZnO and Ag in low density polyethylene (LDPE) packaging, and its influence on chicken breast shelf life and quality.

MATERIAL AND METHODS

Packaging: production and chemical composition

Packaging for chicken breasts, composed of LDPE (LD 654, ExxonMobil, Chemical, USA) blended with a nano-antimicrobial master batch containing Ag and ZnO nanoparticles (Avanzare, Spain) at 0 (control), 5 and 10% w/w, was produced using a JSW J85 ELII electrical injection molding machine, using a clamping force of 85 Ton and a screw diameter of 35 mm. The minimum thickness required for this packaging was determined using Moldflow software (Autodesk, USA). The process variables were optimized using a trial and error process. The final melt temperature was 250ºC, with a profile of 170-190-205-250ºC from the hopper to the nozzle. The mould configuration included a direct conical spurge (diameter 4 mm) and a cooling system with water at 14ºC. The melt was laminated with a screw speed of 99 rpm and a back pressure of 10 bars. Volumetric filling of the mould was programmed for a constant filling time of 0.8 s and a maximum pressure of 570 bars. A holding pressure (up to 15 bars) was applied for 4.2 s to ensure the correct dimensions of the product. The total cycle time was at 26 s after 15 s of in-mould cooling. A total of 100 cycles were

completed for each packaging formulation (LDPE control, 5 and 10% wt. ZnO+Ag) to ensure the repeatability of the process. Table 1 shows the general characteristics of the packaging.

Antimicrobial activity of the packaging

The antimicrobial effect of the nanoparticle additive on Escherichia coli, Pseudomonas aeruginosa and Listeria monocytogenes was investigated using the control LDPE and the LDPE plus 5% ZnO+Ag packaging, following standard ISO-22196:2007. Samples of both packaging types were cut into 5x5 cm pieces and inoculated with 0.4 ml of microbial suspension, performed separately for each microorganism. Estimated initial concentrations were of 2.00E+05 cells/ml for E. coli, 2.50E+05 cells/ml for P. aeruginosa and 7.50E+05 cells/ml for L. monocytogenes. These concentrations were obtained after making decimal dilutions of the initial suspension of the pre-cultured bacteria. The concentration of initial suspension was estimated by microscopy in Thoma camera. Test specimens were then covered with a 4x4 cm piece of sterile film. Immediately after inoculation, half of untreated test specimens were processed for bacteria recovery. The process consisted in adding 10 ml of validated neutralizer and performing 10-fold serial dilutions accordingly to the cited standard. A 100 μL sample from each assay was plated and incubated at 37°C for 24-48 h before counting. The viable bacteria counted for test specimens immediately after inoculation were 3.7 log ufc/cm2 for E. coli, 3.35 log ufc/cm2 for P. aeruginosa and 4.19 log ufc/cm2 for L. monocytogenes. Test specimens with and without treatment were incubated for 24 h at 35ºC. Same process was applied to test specimens after incubation in order to determine the viable bacteria count. The counts for untreated specimen increased as expected and the counts for treated specimens showed values <0.1.

Nanoparticle migration assays

Nanoparticle migration assays were performed using the packaging with the highest concentration of nanoparticles (LDPE plus 10% ZnO+Ag). Analyses were performed using an aqueous food simulant to replace the chicken breasts, at 40ºC over a period of 10 days (EU Regulation 10/2011). The quantification of ZnO and Ag particles in the aqueous food simulant were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7500 ce Octopole Reaction System.

Packaged chicken breasts: packaging type/storage time assays

Freshly skinned and deboned chicken breasts (n=162) were obtained from the processing line at a local poultry plant in northern Spain. These were randomly assigned to one of the three experimental packaging types (LDPE control, 5 and 10% wt. ZnO+Ag). Each package contained two breasts from different chickens. All samples were packed with a standard MAP atmosphere ($O_2/CO_2/N_2$ = 70%/20%/10% respectively). Packages were coded with a three-number code and placed in cold storage at 4ºC under 12 h of light (583 ± 97.2 lux) per day, for 0, 7, 10, 15 or 21 days. To ensure homogeneous light exposure, the packages were randomly moved around the refrigerator over the storage time.

Meat quality testing

Packaging atmosphere

The composition of the atmosphere in the packages after the different storage times was measured using an Oxybabe gas analyzer (WITT-Gasetechnik GmbH & Co KG, Witten, Germany).

Sensorial attributes

Chicken breast samples destined for sensorial analysis (limited to breasts stored for 0 and 10 days given the potential microbiological danger associated with meat stored for longer) were extracted from the test packages, vacuum packed, and frozen at -20ºC until analysis. On the day of evaluation, the still vacuum-packed meat was thawed by immersion in tap water for 4 h until an internal temperature of 17-19ºC was reached (monitored using a Jenway thermocouple attached to a probe). It was then wrapped in aluminum foil and cooked on a pre-heated double hot-plate grill at 200ºC until an internal temperature of 70ºC was attained. The meat was then cut into nine small portions, wrapped in aluminum foil, and stored warm (60ºC) until tasted. Samples were served to a trained (ISO-8586:2012) nine-member-strong panel. Panelists sat in individual booths and under red lighting to mask the color of the meat. Panelists were asked to evaluate the following attributes on a 100-point scale with 1 as the lowest and 100 as the highest score for each attribute: chicken odor intensity, milk odor intensity, cereal odor intensity, tenderness, juiciness, sandy texture, chicken flavor intensity, acid flavor intensity, fat flavor intensity, and off-flavor intensity. The chicken breasts in all packaging types, stored for all other storage times, were visually rated for general appearance by three trained observers on a three point scale (1, bad, 2, good, 3 very good).

Samples from all packaging/storage time treatments were cut into two portions. One of the halves was vacuum-packed and destined for bacteriological enumeration, while the other was checked for color and lipid oxidation.

Microbial counts

For bacteriological enumeration, *Enterobacteriaceae* and mesophiles were counted at 0, 10, 15 and 21 days of storage, whereas *Lactobacillus* were counted at 7, 10, 15 and 21 days. *Enterobacteriaceae* counting was performed following standard ISO 21528-2:2004 (part 2, colony-count method). Samples were homogenized and 10-fold dilutions made in buffered peptone water. A 1 ml sample of each dilution was plated on Violet Red Bile with Glucose (VRBG) agar and incubated at 30ºC for 18-24 h. The number of colony-forming units (cfu) per gram of sample was determined by counting typical colonies. Mesophiles were enumerated following standard ISO 4833:2003 (colony-count technique at 30ºC), this time plating 1 ml of a similar 10-fold dilutions on Plate Count Agar (PCA). After incubation at 30ºC for 72 h, the cfu per gram of sample were determined. For *Lactobacillus* enumeration, 100 µl samples of similar 10-fold dilutions were plated on Man, Rogosa and Sharpe (MRS) agar. After incubation at 35ºC for 3 days, or 30ºC for 5 days, in an aerobic atmosphere supplemented with 5% carbon dioxide, the cfu per gram of sample were determined.

Color variables and visual scores

Breast meat color was examined using a Minolta CM-2600d spectrophometer (CIELAB, D65, 10º, 0% UV, SCI, 8 mm), recording lightness (L*), red index (a*) and yellow index (b*) scores. The Chroma (C*) was then calculated as $C^*=(a^{*2}+b^{*2})^{0.5}$. (AMSA, 1991). Visual scores were daily determined by a six member trained panel (ISO 6658:2005), using a 3 points scale (1, reject, 2, acceptable but not good, 3, good).

Lipid oxidation

After measuring the breast meat color, the samples were vacuum packed and frozen at -20ºC until analyzed by the TBARS method to determine the degree of lipid oxidation suffered (Ripoll *et al.*, 2013). Briefly, meat samples were mixed with trichloroacetic acid and centrifuged, the supernatant removed and the filtrate vortexed with thiobarbituric acid, homogenized, and incubated at 97°C for 20 min in a water bath. The absorbance at 532 nm was then measured. A standard calibration curve was created with increasing concentrations of 1,1,3,3,tetramethoxypropane (99%), the precursor of malonaldehyde (MDA). The final conversion of 1,1,3,3,tetramethoxypropane to MDA was accomplished by multiplying the number of μM of 1,1,3,3, tetramethoxypropane equivalent per gram of sample by the molecular weight of MDA. TBARS values are expressed as milligrams of MDA per kilogram of sample.

Figure 1: The sampling procedure followed.

Statistical analysis

Statistical calculations were generally performed using SPSS 15.0 software. The effect of packaging type and storage time on meat quality variables was analyzed using the general linear model (GLM) procedure. Differences between means were examined using the Duncan test; significance was set at $p<0.05$. The results of the sensorial analysis were examined by generalized procrustes analysis (GPA), which uses translation, rotation and isotropic scaling to minimize differences among panelists (Gower, 1975; Carlucci *et al.*, 1998) (performed using XLStat software). The results are shown graphically in the form of a biplot that includes packaging type and storage time.

RESULTS

Antimicrobial activity of the packaging and migration assays

The antimicrobial activity of the LDPE plus 5% ZnO+Ag packaging, expressed in terms of decimal reduction, was R=7.34 log ucf/cm2 for E. coli, R=6.74 log ucf/cm2 for P. aeruginosa and R=4.31 log ucf/cm2 for L. monocytogenes, i.e., above 4 in all cases. According to Japanese Industrial Standard JIS Z 2801:2000, from which ISO 22196:2007 derives, an antimicrobial activity of R>2.0 log ucf/cm2 is required for the nano food packaging to demonstrate antimicrobial efficacy, as R is de difference in bacteria concentration (expressed in log ucf/cm2) between the non-treated and treated test specimens. Results showed a destruction of 99.99% of inoculated microorganisms.

Nanoparticle migration in the aqueous food simulant was very low. Migration of Zn in control packaging was below the detection limit (<0.005 ppm) whereas in added

packaging, concentrations of just 2.44 ±0.37 ppm of ZnO were detected. On the other hand, the Ag concentration was always below the detection limit (<0.001 ppm). These concentrations are below the maxima allowed (25 mg/kg) by law (R. CE 10/2011). .

Meat quality

Table 2 shows the effect of packaging type and storage time on the meat quality variables examined.

Packaging atmosphere

Figure 2 shows the change in package atmosphere composition over storage time. This variable was clearly influenced by packaging type and storage time, as well as by their interaction. Differences among packages in terms of O_2 were detectable only at 10 days and 21 days of storage, with smaller amounts detected for the control packaging treatment, especially at 21 days of storage. With respect to CO_2, differences among packages were detectable at all times except at 0 days; higher concentrations were recorded for the control packaging type at all storage times. Within packaging types, storage time effects were detectable after 15 days with respect to the O_2 concentration, and at 21 days with respect to CO_2 (with O_2 decreasing and CO_2 increasing with storage time).

Sensorial attributes

Table 3 shows the mean sensorial attribute values recorded. Figure 3 shows the GPA results. Since sensorial testing involved comparisons of all treatment samples, results are shown in terms of combined packaging type/storage time. Meat in the LDPE plus 5% and 10% ZnO+Ag packaging stored for 0 days had lower cereal odor and tenderness scores than the rest, meat in the LDPE plus 5% ZnO+Ag packaging had the least sandy texture. Meat in the LDPE plus 10% ZnO+Ag packaging stored for 0 days returned lower values for cereal odor intensity than meat in the same packaging stored for 10 days. In packaging made from either LDPE plus 5% or 10% ZnO+Ag, meat stored for 0 days returned lower values for tenderness than after storage for 10 days. In GPA (Fig. 3), Axis 1 explained 38.59% of the variation in the relationships between treatments and sensorial attributes, whereas Axis 2 explained 22.26%. All samples stored for 10 days fell to the right of the graph, while those stored for 0 days fell to the left, except for the control packaging samples which took a position among those stored for 10 days. Samples in the LDPE plus 5% ZnO+Ag packaging took a position at the top half of the graph, separated from the others.

Microbial counts

Figure 4 shows the microbial counts for the different packaging types over storage time. As expected, counts for all types of bacteria increased with storage time, independent of the packaging type. In general, all microbial counts were higher in the control packaging treatment at all storage times. With respect to mesophiles, the differences between packaging types were not very great and usually detectable only at 15 days. For *Enterobacteriaceae*, differences were detectable at 10 and 15 days of storage, but not at 0 days when counts were very low, nor at 21 days when they were very high. With respect to *Lactobacillus*, differences between packaging types were found only at 21 days.

Color variables and visual scores

Table 4 shows the color variable and visual scores with respect to packaging type and storage time. Meat stored for 21 days was sticky due to spoilage; color measurements were therefore likely to be inaccurate and the data are not shown. No differences were seen between packaging type in terms of meat color or visual appearance score, then, the results showed in Table 4 are a mean of the values obtained for the thee essayed packaging.

Redness (a*), yellowness (b*) and Chroma (C*) reached maximum values at 7 days and descended thereafter, whereas lightness (L*) reached its maximum at 15 days of storage. Visual scores fell continuously from the first to the last day of storage, especially after 10 days.

Lipid oxidation

Lipid oxidation (Fig. 5) increased with storage time for all packaging types. Differences between packaging types were detectable at 10 and 21 days of storage. The control packaging returned the lowest TBARS value at the beginning of storage.

DISCUSSION

Nanoparticle migration

Huang *et al.* (2011) report the migration of Ag nanoparticles from commercially available packaging into food-simulating solutions under incubation conditions similar to those used in the present work. However, the packaging studied by the latter authors contained more Ag (0.01% compared to the present 0.008%). In the present work, ZnO migration was more common than Ag migration, probably because the ZnO concentration of the packaging packages was higher (0.12% compared to 0.008%). Even so, the amount of ZnO that migrated to the food was well below the limit established by EU Regulation 10/2011 (25 mg/kg food or food simulant).

Changes in package atmosphere

High-oxygen packaging atmospheres are commonly used in the poultry meat industry; package atmospheres are typically 70% O_2, 20% CO_2 and 10% N_2 (Fraqueza and Barreto, 2011). However, growing bacteria consume mainly O_2, CO_2 dissolves in the water of meat, and both O_2 and CO_2 escape through the barrier film at different rates (Brown, 1992). Therefore, under commercial conditions, the CO_2 concentration of the atmosphere remains fairly constant, whereas the O_2 concentration decreases with storage time (Gill, 1996). In the present work, the depletion of oxygen and the increase in CO_2 were significant after 10 days of storage, irrespective of the packaging type. However, these changes were most noticeable with the control packaging; the reduced microbial counts in the treated packaging treatments likely led to low O_2 consumption (Rotabakk *et al.,* 2006).

Effects on meat sensorial attributes

The lack of effect of packaging type or storage (at least up to the maximum 10 day testing time) on flavor and odor attributes agrees with that reported by authors (Lyon *et al.*, 2001; Liu *et al.*, 2004; Zhuang *et al.*, 2007; Zhuang and Savage, 2010). According to Northcutt *et al.* (2001), few factors during production and processing

affect poultry meat flavor. However, Smolander *et al.* (2004) reported changes related to storage time and temperature.

Many authors report an effect of storage time on tenderness and other textural attributes (Lyon *et al.*, 2001; Zhuang *et al.*, 2007; Zhuang and Savage, 2010). Liu *et al.* (2004) performed a principal components analysis (PCA) with 24 variables and reported the Warner-Bratzler shear force to be strongly correlated with five sensory texture attributes, but insignificantly correlated to flavor and after-feel. Zhuang and Savage (2010) studied the relationships between color and the sensory profile of chicken breast fillets, and found a positive relationship between L* and hardness, cohesiveness, chewiness, and the rate of breakdown. Finally, Lyon *et al.* (2001), also via PCA, found that the 17 attributes they examined were explained in terms of four factors: texture, moisture, chickeny-meaty and off-flavor. The present Figure 3 shows a distribution of attributes very similar to that reported by Lyon *et al.* (2001).

All the sensorial attribute scores fell in the mid part of the 1-100 intensity scales, except for milk odor intensity, which scored in the low part of the scale, and tenderness which scored in the higher part. The ranges recorded for the different scores were slightly wider than those reported by other authors (Lyon and Lyon, 1997; Liu *et al.*, 2004; Zhuang and Savage, 2010). The current coefficients of variation for sensorial attributes agree with those of Zhuang *et al.* (2007), who reported values of around 20% for chicken odor and tenderness, 40% for sour flavor, and more than 100% for fat flavor. However, the present coefficients were larger than those reported by Zhuang and Savage (2010); these authors recorded coefficients of variation of <20% for all attributes. In other species, coefficients of variation of around 30% have been reported (Campo *et al.,* 1999).

Microbial counts

The present mesophile, *Enterobacteriaceae* and *Lactobacillus* counts agree with those reported by other authors. Smolander *et al.* (2004) reported lactic acid bacteria (which includes *Lactobacillus*) counts of around 10^4 cfu/g at the beginning of storage, rising to log 7 over the first 7 days of storage, and *Enterobacteriaceae* counts of around 10^2 at the beginning of storage rising to 10^6-10^8 cfu/g after 12 days. Similarly, Voidaru *et al.* (2011) reported counts of 4.7 log cfu/g for total aerobic bacteria, whereas Álvarez-Astorga *et al.* (2002) described 5.79 log cfu/g for mesophiles and around 3.56 log cfu/g for coliforms in chicken legs.

In the European Union, chicken meat is required to be free of *Samonella* (measured in 25 g samples) (EU Directives 1441/2007 and 1086/2011). Given the absence of other microbiological standards, Pascual-Anderson (1992), who established guideline limits for chicken carcass contamination, recommended the number of mesophiles (which includes *Salmonella*) to be no greater than 6 log cfu/g. However, Sánchez *et al.* (2011), who measured microbial counts in chicken breast meat in a commercial slaughterhouse over a period of one year, reported a mean value of around log 3.65 cfu/g for aerobic bacteria (which includes mesophiles), and around log 2.61 cfu/g for *Enterobacteriaceae*. Consequently, these authors established a recommended limit of log 4.84 cfu/g for aerobic bacteria and of log 3.70 cfu/g for *Enterobacteriaceae*. These limits agree with those suggested by other authors (between log 6 and log 7 for aerobic bacteria and log 2 and log 3 for *Enterobacteriaceae*) (Wehr 1982, Sumner, 2004, Smolander *et al.*, 2004). The present results for mesophiles were below the limits established by Sánchez *et al.* (2011) for the first week, and within the log 7 limit

suggested by Smolander *et al.* (2004). Counts for *Enterobacteriaceae* were below the recommendations described by Sánchez *et al.* (2011) until 5 or 10 days of storage, depending on the packaging. Finally, *Lactobacillus* counts for all packaging types and at all storage times were very low compared to those reported in the literature (Smolander *et al.*, 2004).

The chemical composition of the packaging had a clearer effect on *Enterobacteriaceae* than on mesophiles or *Lactobacillus*. Since the differences in chemical composition of the packaging lead differences in atmosphere composition, these results, mainly CO_2 amount, present results would agree with other authors' reports that an increase in the CO_2 concentration inhibits the growth of *Enterobacteriaceae* (Fraqueza and Barreto, 2011), and that lactic acid bacteria are less affected by the package atmosphere composition than are aerobic bacteria (Gill, 1996; Rotabakk *et al.*, 2006)

Color and visual scores

Color is the primary attribute influencing consumer choice of chicken meat (Barbut, 2001). The present results for all packaging types and storage times agree with those of Anang *et al.* (2010), who described values of around 50 for L*, from 2.93 to 5.54 for a* and from 3.92 to 8.85 for b* for chicken breast stored at 4ºC for 0 to 14 days. Other authors have reported similar values (Allen *et al.*, 1998; Fletcher *et al.*, 2000; Fanatico *et al.*, 2007). Qaio *et al.* (2002) classified chicken breast quality with respect to color, reporting color values for normal breasts to be 62.07 for L*, 4.38 for a* and 9.68 for b*. Allen *et al.* (1998) described fillets with L* values of up to 50 as "light", while below this value they scored them as "dark". The present L* values were 50 or above at all times in all treatments; hence, these samples can be considered "light". Despite the differences found in atmosphere composition, no differences in color were found between packaging, which was in agreement with Rotabakk *et al.* (2006) who described no differences in color due to differences in package CO_2 concentration.

The present results for all treatments were associated with coefficients of variation of 5.0% for L*, 59.1% for a*, and 51.7% for b*. The coefficients of variation reported by Allen *et al.* (1998) and Fletcher *et al.* (2000) were very low for L* (1.9 and 4.2 respectively) but slightly higher for a* (around 18 in both studies), and quite high for b* (29 and 28 respectively). Nevertheless, Qaio *et al.* (2002) reported much higher coefficients: 20% for L*, 100% for a* and 54% for b*.

In beef, it has been described that C* values above 18 represent a bright color well accepted by consumers (MacDougall, 1982); below this value the meat has a dull color that could lead to rejection. C* values for chicken meat are usually between 2 and 12, largely depending on pre-slaughter conditions and handling practices (Allen *et al.*, 1998). In the present work, C* was always under 4. Data suggest that during the period in which L* and C* are both increasing its values, meat is visually acceptable. However, since the moment in which this trend changes, which in our study occurs from day 10, the combination L*high values-C* low values, leads a decrease of visual scores, and finally, meat rejection. More studies are needed, using different types of broilers, to determine the critical relationship between variables and to establish a reference threshold of reference.

Lipid oxidation index

Lipid oxidation (Fig. 5) increased with storage time irrespective of packaging type, although both types of packaging with nanoparticles generally returned lower lipid

oxidation values. This suggests that the lower antimicrobial counts associated with the treated packaging influenced lipid oxidation. It is generally accepted that oxidation increases with air-exposure time (De Smet *et al.*, 2008; Luna *et al.*, 2010; Narciso-Gaytán *et al.*, 2010). As reported by Gatellier *et al.* (2007), poultry meat contains relatively high levels of unsaturated fatty acids and low levels of natural antioxidants, and is therefore particularly prone to oxidation. In beef, a critical value of 2 mg/g MDA is accepted as the threshold of detection by consumers (Campo *et al.*, 2006), but in poultry this threshold is much lower and varies depending on diet composition and exercise (Veberg *et al.*, 2006). De Smet *et al.* (2008) describe values from 0.07 µg/g to 0.52 µg/g, from 0.14 µg/g to 0.77 µg/g, and from 0.09 µg/g to 0.72 µg/g in chicken meat stored for 3, 7 and 10 days respectively. Gatellier *et al.* (2007) reported values from 0.1 to 0.5 µg/g in chicken breast, and Veberg *et al.* (2006) values of 0.32 µg/g in minced turkey meat. The present results agree with these figures. According to Gatellier *et al.* (2007), the amount of malonaldehyde remains fairly constant during the 4 first days of air exposure, and increases thereafter until day 9, in agreement with the present results.

In conclusion, adding ZnO+Ag nanoparticles to LDPE packaging had an antimicrobial effect both *in vitro* and on meat. Such packaging additives would appear to be safe since they migrate into the food in amounts well within those allowed by law. Regarding meat quality, packaging added ZnO+Ag nanoparticles showed a lower depletion of oxygen and lower microbial counts than control packaging. Sensorial attributes were slightly affected by packaging and no differences were seen between packaging type in terms of meat color or visual appearance score. The addition of ZnO+Ag nanoparticles to LDPE packaging delayed breast spoilage and lipid oxidation

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Table 1. General properties of the packaging material.

Property	**Test method**[a]	**Value**
Melt index, g/10 min	D 1238	70
Density, g/cm^3	D 1928/1505	0.913
Melting point, ºC	D 3418	100
Crystallization point, ºC	D 3418	84
Vicat softening point, ºC	D 1525	75
Mechanical properties[b]		
Tensile strength at break, MPa	D 638	8
Elongation at break, %	D 638	100
1% secant modulus, MPa	D 638	90
Shore hardness-D (15 s)	D 2240	41

[a] ASTM methods

[b] 3 mm-thick type I injection molded specimen, according ASTM D 638

Figure 1. Experimental design.

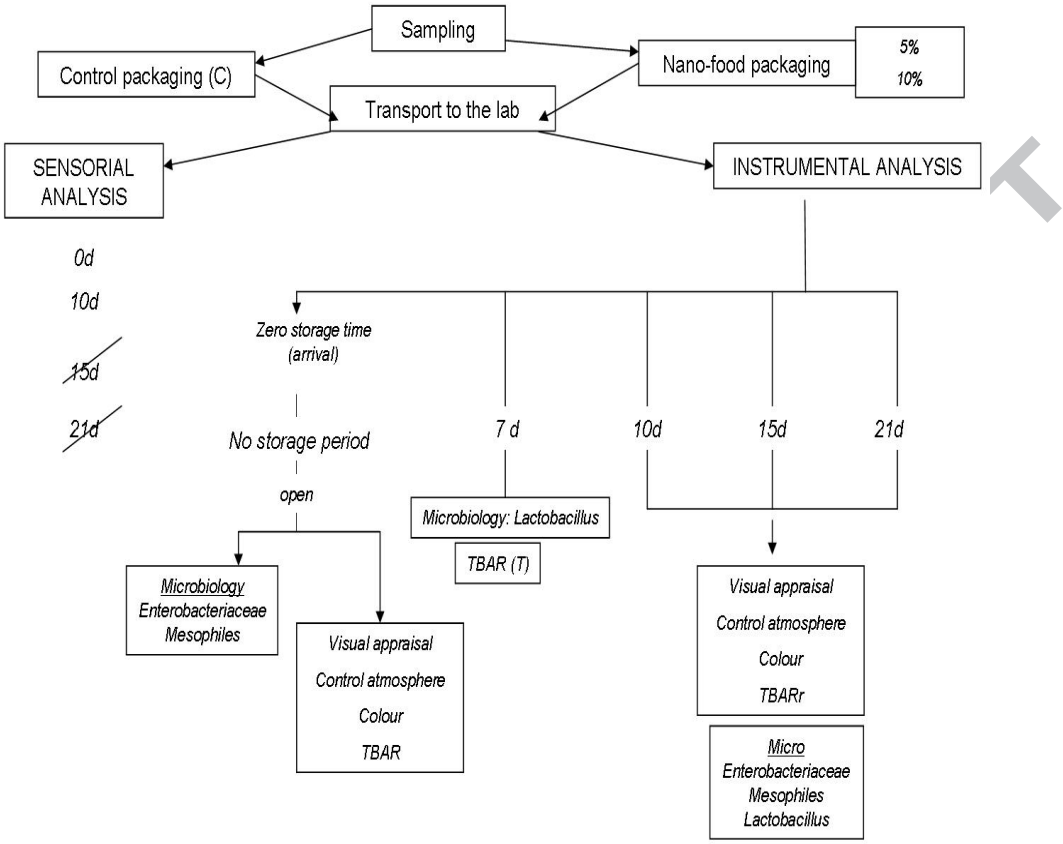
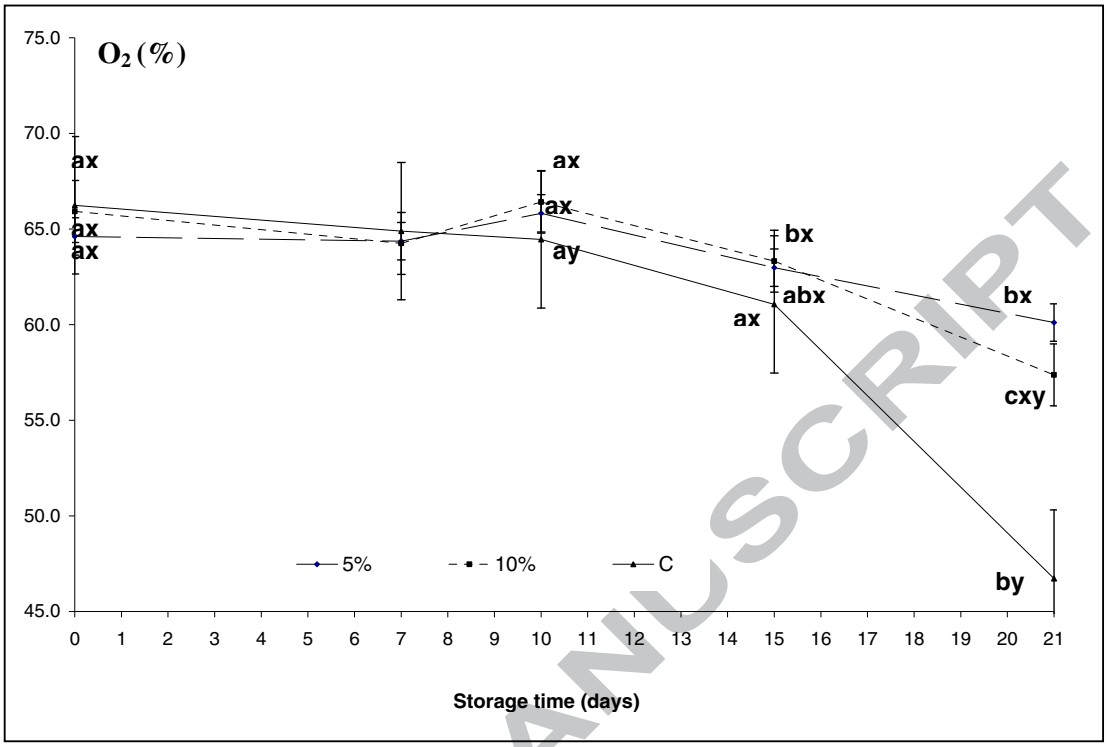
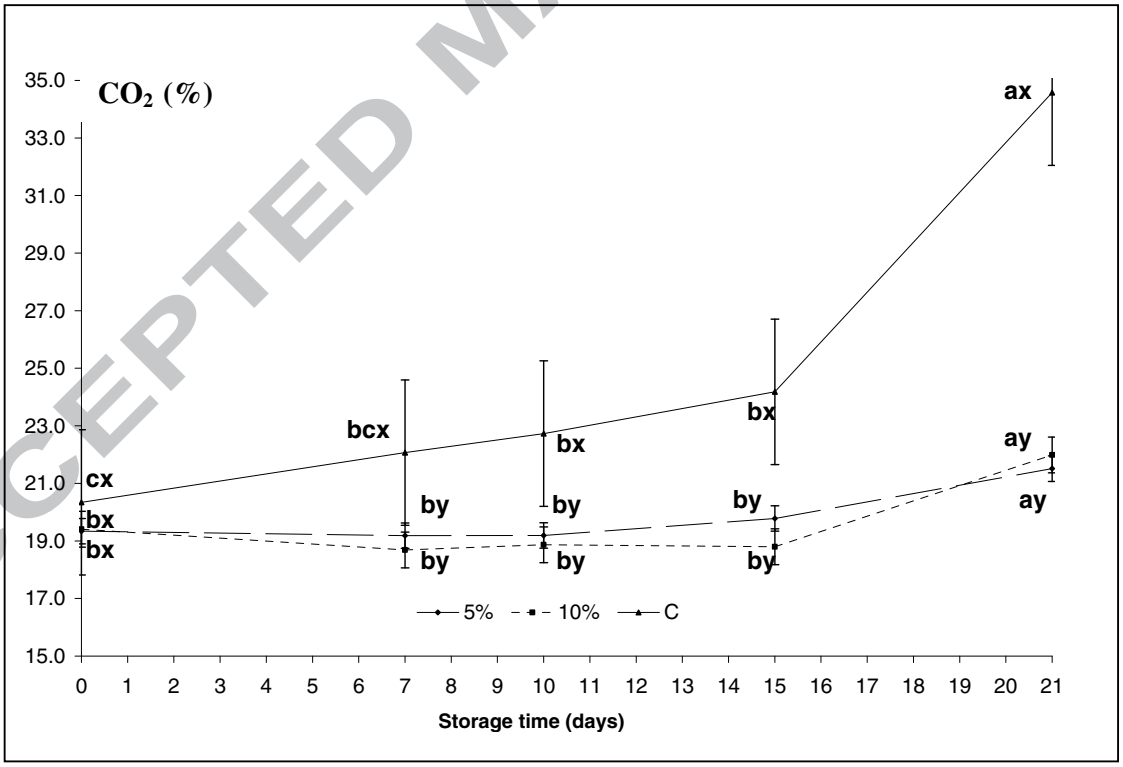


Table 2. Significance (P values) of the influence of packaging type and storage time, and their interaction, on package atmosphere composition, microbial numbers and meat quality.

	Pack composition (C)	Storage time (T)	*C*T*
Atmosphere			
O_2	0.004	0.000	*0.001*
CO_2	0.000	0.000	*0.000*
Sensory analysis			
Chicken odor intensity	0.139	0.400	*0.548*
Milk odor intensity	0.658	0.081	*0.611*
Cereal odor intensity	0.178	0.037	*0.488*
Tenderness	0.501	0.000	*0.012*
Juiciness	0.628	0.259	*0.252*
Sandy texture	0.007	0.388	*0.175*
Chicken flavor intensity	0.252	0.376	*0.633*
Acid flavor intensity	0.690	0.556	*0.964*
Fat flavor intensity	0.642	0.773	*0.995*
Off flavor intensity	0.889	0.849	*0.493*
Microbiology			
Enterobacteriaceae	0.000	0.000	*0.001*
Mesophiles	0.000	0.000	*0.029*
Lactobacillus	0.028	0.000	*0.569*
Instrumental analysis			
L*	0.800	0.000	*0.086*
a*	0.568	0.025	*0.007*
b*	0.576	0.006	*0.346*
H^0	0.503	0.354	*0.006*
C*	0.722	0.002	*0.534*
Color (visual appraisal)	0.170	0.000	*0.262*
Lipid oxidation	0.050	0.000	*0.341*

Figure 2. Change in package atmosphere over storage for different packaging types.





x, y and z reflect significant differences (P<0.05) between packages within a storage time.

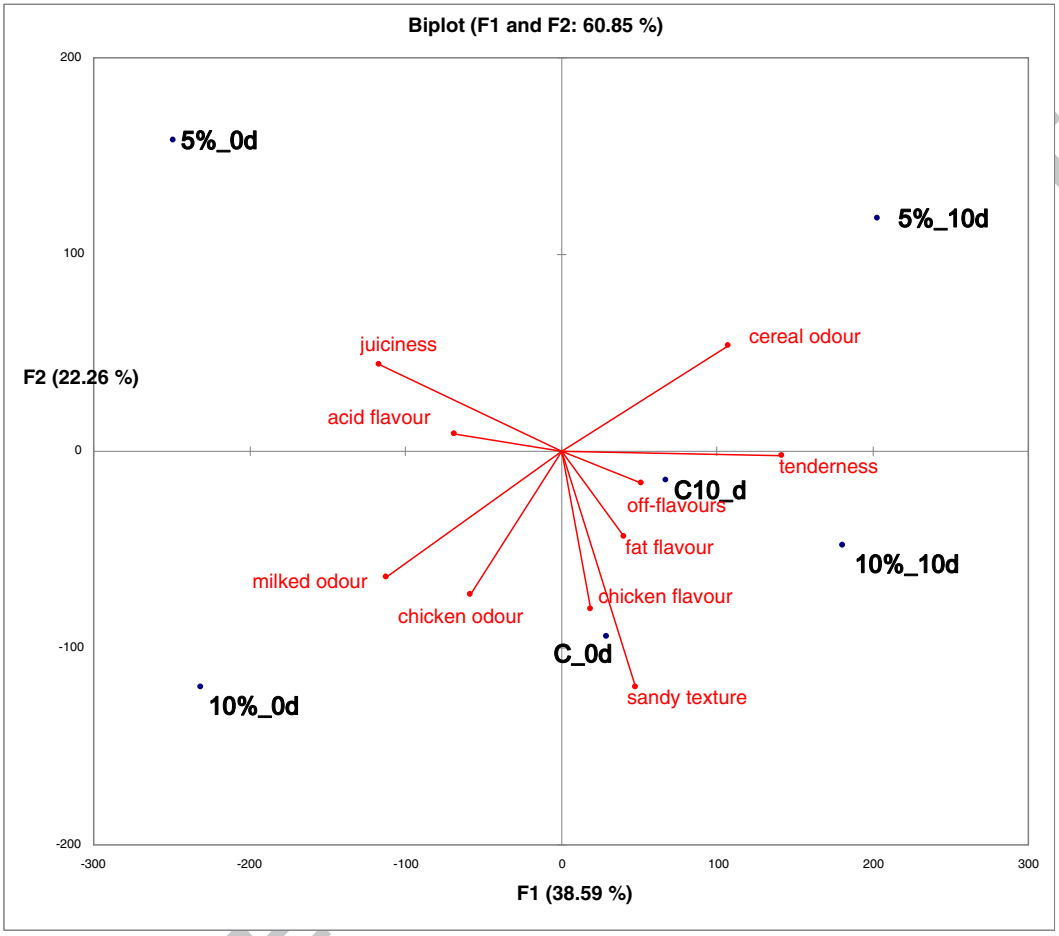
a, b, c and d reflect significant differences (P<0.05) between storage times within a packaging type.

Table 3. Values for chicken breast sensorial attributes over storage time (T) with respect to different packaging type.

	LDPE plus 5% ZnO+Ag		LDPE plus 10% ZnO+Ag		LDPE plus 0% ZnO+Ag (control)		s.e.
Storage time	0 d	10 d	0 d	10 d	0 d	10 d	
Odor intensities							
Chicken	54.89	50.84	55.41	54.30	57.18	57.68	2.237
Milk	25.29	21.48	28.25	20.86	26.68	24.61	2.975
Cereal	39.97 ^{ab}	43.32 ^{a}	31.45	42.91 ^{a}	42.84 ^{b}	44.80 ^{b}	3.495
Texture attributes							
Tenderness	56.97 ^{b}	66.79 ^{a}	55.07^{b}	71.79 ^{a}	65.20 ^{a}	66.50 ^{a}	9.156
Juiciness	55.20	46.84	51.05	50.09	47.77	48.98	2.887
Sandy texture	39.66 ^{b}	48.74 ^{ab}	56.07 ^{a}	53.07 ^{a}	55.34 ^{a}	56.86 ^{a}	3.671
Flavor intensities							
Chicken	55.84	56.98	60.34	59.98	56.05	60.20	2.255
Acid	42.42	40.16	40.89	39.88	43.36	41.80	3.489
Fat	38.49	38.91	38.39	39.49	41.09	41.95	3.484
Off flavors	36.67	42.59	39.51	40.50	40.45	36.09	4.045

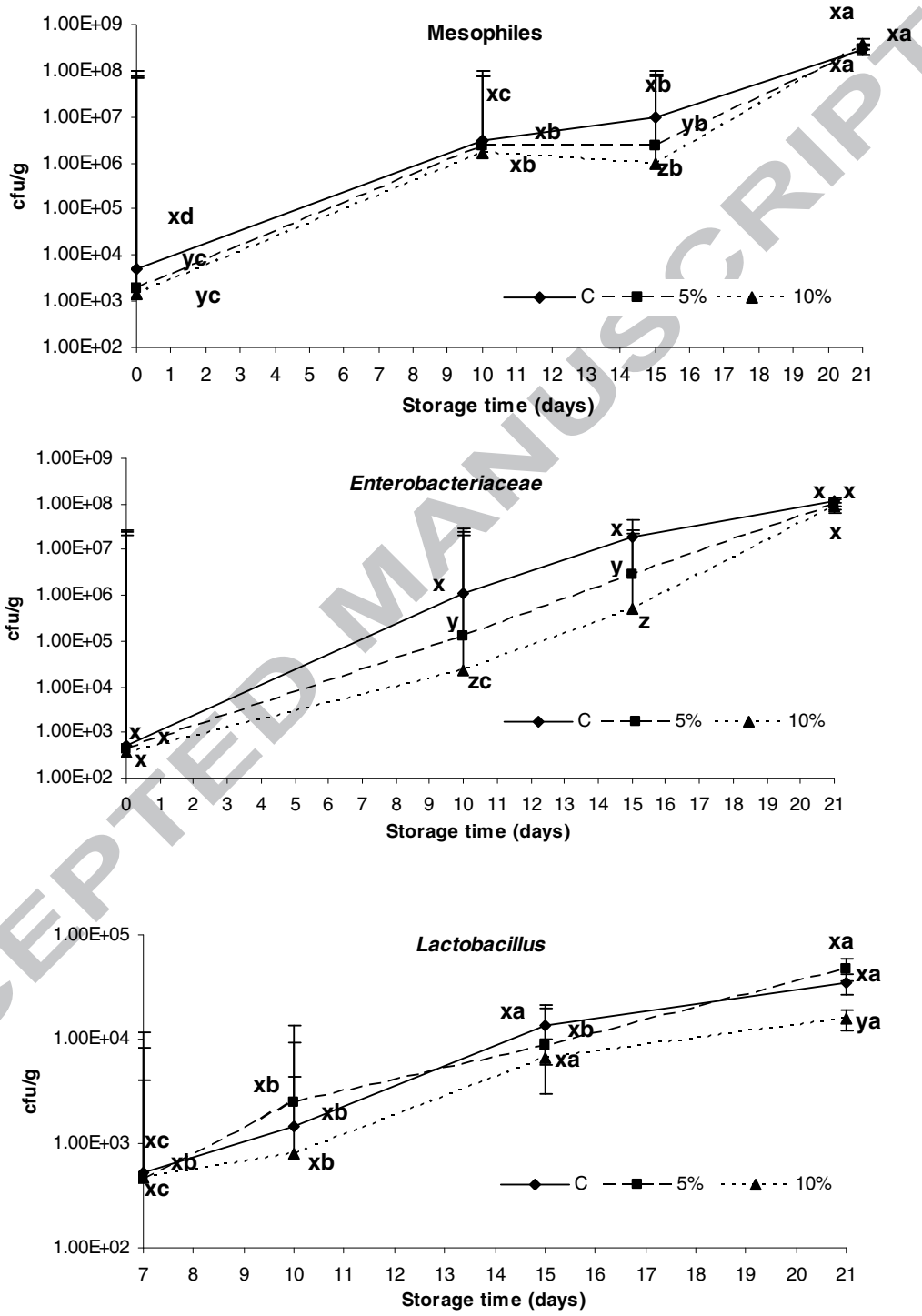
s.e.- mean standard error
Superscripts 'a' or 'b' in the same row reflect significant differences (P<0.05) between treatments (combination pack/storage time)

Figure 3. Biplot derived from generalized procrustes analysis of chicken breast sensory traits over storage time with respect to packaging type.



5% = LDPE plus 5% ZnO+Ag, 10% = LDPE plus 10% ZnO+Ag, C = LDPE plus 0% ZnO+Ag (control)

*Figure 4. Total mesophiles, Enterobacteriaceae and Lactobacillus (*cfu/*g) in the chicken breasts over storage time in each type of packaging.*



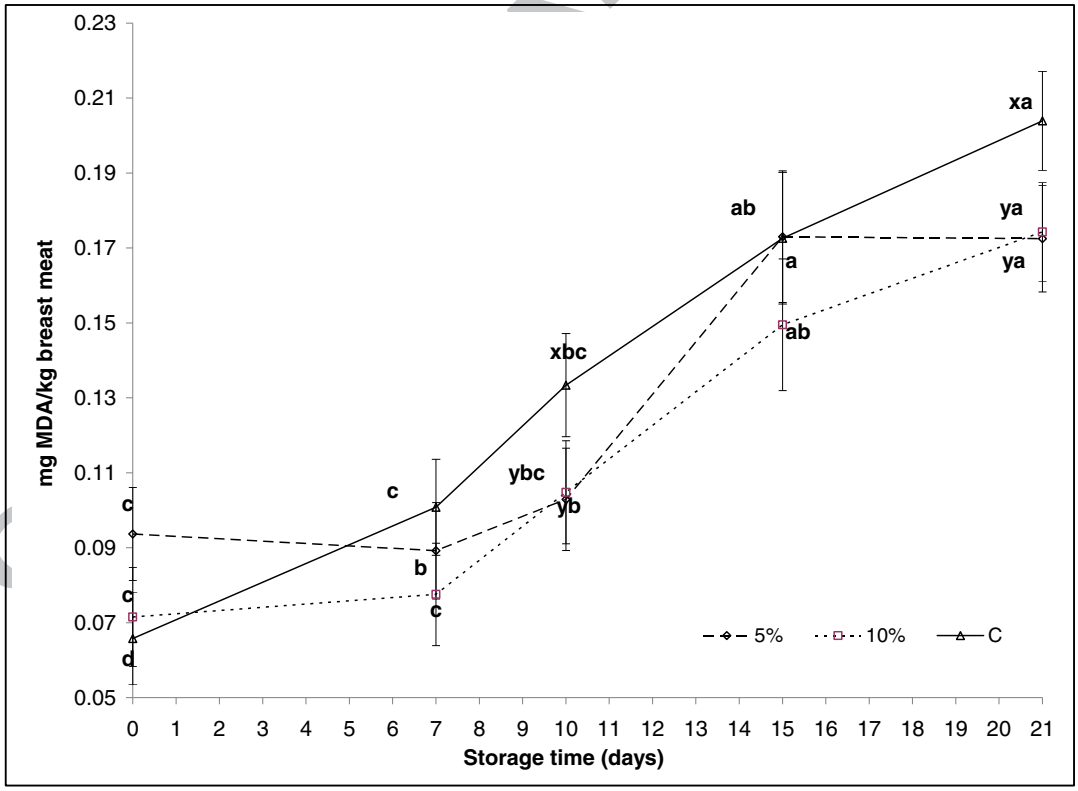
x, y and z reflect significant differences (P<0.05) between packaging types within a storage time. a, b, c and d reflect significant differences (P<0.05) between storage times within a packaging type.

Table 4. Change in color and visual score over storage time. Since packaging has no effect on color and visual scores, showed values are a mean of the values obtained for the three essayed packaging.

	L*	a*	b*	C*	Visual scores
0 days	50.4 c	0.8 b	1.8 b	2.0 b	3.0 a
7 days	54.0 b	1.4 a	3.4 a	3.7 a	3.0 a
10 days	53.5 b	1.1 ab	2.0 b	2.4 b	2.5 b
15 days	55.9 a	0.9 b	2.4 b	2.8 b	1.5 c
21 days	-	-	-	-	1.0 d
P value	0.000	0.038	0.001	0.000	0.000
s.e.	0.35	0.81	0.16	0.15	1.05

s.e.- mean standard error
a, b, c and d reflect significant differences (P<0.05) between storage times.

Figure 5. Lipid oxidation of chicken breast meat in different packaging types after



different storage times.
x, y and z reflect significant differences (P<0.05) between packaging types within a storage time.
a, b, c and d reflect significant differences (P<0.05) between storage times within a packaging type.