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Effect of a Chitosan-Based Packaging Material on the Domestic Storage of "Ready-to-Cook" Meat Products: Evaluation of Biogenic Amines Production, Phthalates Migration, and In Vitro Antimicrobic Activity's Impact on Aspergillus Niger

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Abstract: The consumption of "ready-to-cook" foods has been experiencing rapid expansion due to modern lifestyles, and they are often sold in economical multipacks. These foods necessitate packaging that maintains their quality for extended periods of time during home storage once the original packaging is opened. This study evaluates a chitosan-based film derived from low- and high-molecular-weight (MW) chitosan in acetic acid without synthetic additives as an alternative packaging material for "ready-to-cook" beef burgers. The burgers were stored at 8 °C after being removed from their sales packaging. A commercial polyethylene (PE) film designed for food use, devoid of polyvinylchloride (PVC) and additives, served as the reference material. The production of six biogenic amines (BAs), indicative of putrefactive processes, was monitored. Additionally, the release of four phthalates (PAEs), unintentionally present in the packaging films, was assessed using solid-phase microextraction coupled with gas chromatography/mass spectrometry (SPME-GC/MS). Microbiological tests were conducted to investigate the antimicrobial efficacy of the packaging against Aspergillus Niger NRR3112. The results showed that the chitosan-based films, particularly those with low MW (LMW), exhibited superior meat preservation compared to the PE films. Furthermore, they released PAEs below legal limits and demonstrated the complete inhibition of fungal growth. These findings highlight the potential of chitosan-based packaging as a viable and effective option for extending the shelf-life and maintaining the quality of "ready-to-cook" meat products during domestic storage.

Keywords: chitosan-based film; food packaging; meat; "ready-to-cook"; SPME-GC/MS; BAs; PAEs; antimicrobial activity

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1. Introduction

Modern lifestyles have changed consumers' eating habits, with a preference for fully or partially prepared foods over traditional home-cooked meals. These products are often sold in more economically advantageous "family packs" to be consumed within a short time after opening. Among these, a significant portion is based on meat, a perishable food whose shelf life depends on many variables such as microbial growth, enzymatic activity, oxidation processes, and package type [1]. This makes processing methods and protective packaging [2,3] that preserve quality for a long time highly necessary.

However, after purchase by the final consumer, meat preservation is mostly influenced by the storage temperature and duration in domestic refrigerators, which often maintain temperatures not lower than 8–10 °C. An indicator of meat quality relies on the evolution of biogenic amines (BAs) [4–6]. These BAs may indirectly provide quality-related information, serving as a sign of poor meat storage. BAs are nitrogenous compounds present in several foods, with varying percentages influenced by the profile of free amino acids, the

microbiological quality, and hygienic measures during food processing [4]. Some of these amines have toxic effects, and their presence represents serious harm to consumers [5–7]. Due to its high amino acid content [5], BAs are widely present in meat, including histamine, tyramine, and polyamines such as cadaverine and putrescine.

In addition to proper storage, the choice of packaging material plays a crucial role in preserving meat products [8]. The food industry has used polymeric materials for wrapping meat products for the past 50 years. Additives are commonly used during the production of packaging materials. For instance, phthalic acid esters (phthalates, PAEs) are added to soften plastics at room temperature. These are widely recognized as dangerous to human health, being endocrine disruptors with potentially carcinogenic, teratogenic, and mutagenic properties [9,10]. Food packaging contributes almost 50% of the total weight of global plastic waste production [11]. Since PAEs are only physically linked to the polymer, they can be easily released into the environment, posing risks as non-intentionally added substances (NIASs) [12], even in packaging without additives such as PE packaging [12]. Consequently, they can be present in the foods they contain, representing a serious safety risk for consumers, as their presence may be unknown even to the manufacturer. Regulation EU No. 10/2011 includes the "Plastic Implementation Measures" (PIM), providing guidelines for assessing the risks of substances migrating from packaging to food and setting the limit for the sum of PAEs to $\leq$60 mg/kg of food product [13].

Furthermore, to extend the shelf-life of meat and meat derivatives, the industry commonly uses a food contaminant, the fungus Aspergillus Niger, capable of producing citric acid, a significant food preservative [14,15]. This fungus also produces vitamins, proteases (such as acetyl esterase, amylase, glucose oxidase, glucosidase, phospholipase, etc.), and chitosan, which are widely utilized as bio-preservative additives [16,17]. However, some strains of Aspergillus can cause severe human diseases due to the production of toxic secondary metabolites [18].

Recently, several packaging materials and industrial approaches have been used to produce alternative eco-friendly packaging materials [19–22], including chitosan, a polymer derived from chitin, largely present in the exoskeletons of crustaceans, insects, and fungi [23–25], which is edible, cheap, and biodegradable. Chitin is the second most abundant polysaccharide on Earth [26], and it can be easily purchased from shell waste in the fish industry, providing a stable renewable source [27]. Chitosan is a natural cationic polysaccharide that easily forms additive-free biodegradable films [28]. Chitosan films are used for food oxide [29] and microwave [30] techniques.

Based on the above considerations, chitosan is of great interest for the scientific community, even if, in certain conditions, it shows poor solubility and low antimicrobial activity compared to other natural bioactive polymers (e.g., pectine, alginate, and amido) [31].

In this study, an alternative chitosan-based food packaging polymer (at low and high molecular weight), free of synthetic additives, was produced using the solution casting method [32,33]. For the first time, it was evaluated on "ready-to-cook" beef burgers after the removal of the original sales packaging under home storage conditions and compared to a commercial polyethylene (PE) film designed for food use. Many articles are present in the literature regarding the effect of chitosan-based films on the preservation of "ready-to-cook" meat as produced [34–37]. To the best of our knowledge, no work dealing with the study of the effect of chitosan packaging on the preservation of "ready-to-cook" meat and meat products' properties after the removal of the original sales packaging in domestic storage conditions has been published so far. The production of six biogenic amines (BAs), indicative of putrefactive processes, was monitored. Additionally, the release of four PAEs, unintentionally present in the packaging films, was assessed using solid-phase microextraction coupled with gas chromatography/mass spectrometry (SPME-GC/MS). Finally, microbiological tests were conducted to investigate the antimicrobial efficacy of the packaging against Aspergillus Niger NRR3112.

2. Materials and Methods

2.1. Chitosan-Based Film Preparation

Chitosan films were prepared using the casting method, following the protocol applied by Leceta et al. [33]. Two types of chitosan, with low molecular weight (LMW; viscosity of 20–200 cP) and high molecular weight (HMW; viscosity of 800–2000 cP), both with a degree of deacetylation higher than 75%, were employed for film preparation.

Briefly, a 1% (*w*/*w*) chitosan solution (Sigma Aldrich, St. Louis, MO, USA) in 1% acetic acid (Sigma Aldrich) was prepared. After 15 min of continuous stirring, glycerol (GLY; Sigma Aldrich; food grade) was added as a plasticizer at a content of 15% (*w*/*w*) of the chitosan. Stirring was continued for 30 min until the mixture was completely homogenized. Subsequently, the solutions were filtered, and 25 mL of the solution was poured into each Petri dish and allowed to dry at room temperature.

All films were then stored for 48 h in a controlled environment chamber (Thermo Fisher Scientific, Waltham, MA, USA) at 25 °C and 50% relative humidity (RH).

2.2. Chitosan-Based Film Characterization

2.2.1. Transmittance Measurements

Regular transmittance (T) at an 8° angle of incidence was measured for chitosan-based and commercial PE films in the 300–1000 nm range using unpolarized light by means of a Varian Cary 500 spectrophotometer (Agilent, Santa Clara, CA, USA). All measurements were repeated in triplicate.

2.2.2. Water Vapor Transmission Rate (WVTR) Measurements

The WVTR of chitosan-based films was measured using a 7002-water vapor permeation analyzer (Illinois Instruments, Inc., Johnsburg, IL, USA). The instrument was equipped with a Pb_2O_5 sensor and displayed the WVTR in units of $g/m^2/day$. According to Faraday's laws, the electrolytic current is a measure of the rate at which water is electrolyzed, which, at equilibrium, is equivalent to the rate at which the Pb_2O_5 film absorbs moisture. The absolute humidity in the sample was determined using the gas flow rate through the housing and the current in the cell. Films were stored in the cell at 37 °C and 90% RH for 24 h.

2.3. Meat Samples

"Ready-to-cook" Beef burgers (2 burgers, each weighing about 100 g, contained in the same double pack under vacuum, and stored at approximately 4 °C) within the expiry date were purchased from a local supermarket. The packaging was intact, and the "cold chain" was respected during transport. The burgers were accurately mixed and then cut with a sterile knife to obtain samples of approximately the same weight (~2.5 g).

Each meat sample was wrapped in industrial PE and chitosan-based films, kept in a domestic refrigerator at 8 °C, and stored for different durations (2, 6, and 9 days). Beef samples were taken immediately after opening the sales packaging (t0) and after storage and processed for the determination of BAs and PAEs using SPME-GC/MS. All samples were prepared in triplicate.

Meat pH Measurements

pH measurements were conducted using a calibrated pH meter (Hanna Instruments, Padova, Italy) on 0.25 g of beef burger homogenized in 10 mL of distilled water under constant stirring. Measurements were taken on the as-sampled meat (t0) and for each selected storage time using the different types of protective films considered.

2.4. BAs Determination

2.4.1. Chemicals

Six BA standards (butylamine, cadaverine hydrochloride, isobutyl amine, isopentyl amine, putrescine dihydrochloride, tyramine hydrochloride) and one internal standard (IS;

1,7-diaminoheptane) were used. All reagents were purchased from Sigma Aldrich and were >99% pure, except for cadaverine hydrochloride and the IS (98%). Isobutyl chloroformate (IBCF; Sigma Aldrich) was used as a derivatizing agent.

Stock solutions of BAs (1 mg/mL) were prepared in sterile filtered ultrapure water (SFUW, Sigma Aldrich) and stored in a refrigerator at 8 °C. Working solutions of analytes alone or in a mixture were prepared daily by diluting stock solutions with SFUW. The IS solution was prepared at a concentration of 0.2 mg/mL and stored at 8 °C for the duration of the trials.

2.4.2. SPME-GC/MS Protocol

The SPME device (Sigma Aldrich) consisted of a manual holder and a silica fiber coated with a polyacrylate (PA) film (diameter 85 µm). Prior to analysis, as instructed by the supplier, the fiber was conditioned in the GC injector. Subsequently, it was exposed for 40 min in a 1.7 mL clear glass vial sealed with a PTFE (polytetrafluoroethylene) septum containing a Teflon-coated magnetic stir bar (length 10 mm, diameter 4 mm) (Sigma Aldrich), following the method of Aresta et al. [38].

In brief, 0.05 mL of standard solutions or supernatant from homogenized and centrifuged meat samples (0.25 g of beef burger in 3 mL of 0.3% trichloroacetic acid, TCA) were transferred into the sealed vial, which was then filled with 1.43 mL of sterile 15% NaCl solution. An IS (7.5 µL) was added using a syringe, and the pH of the resulting solution was adjusted to 12 with 4 N NaOH (6.0 µL). Finally, 7.5 µL of the derivatizing agent (IBCF) was added. The vial was manually shaken for 2 min at room temperature, and the SPME fiber was exposed to the solution. The fiber was then carefully removed from the vial and directly inserted into the GC injector for 10 min for GC/MS analysis.

The GC/MS system used was a Finnigan TRACE GC ultra gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a split/splitless injector interfaced with an ion trap MS (Finnigan Polaris Q, Thermo Fisher Scientific). The capillary column used was a Sigma Aldrich SPB-5 fused silica (30 m length, 0.25 µm i.d., 0.25 µm film thickness) with helium (purity > 98%, Rivoira, Bari, Italy) as the carrier gas at a flow rate of 1 mL/min. The transfer line temperature was set at 300 °C, while the injector (in splitless mode for 1 min) was maintained at 250 °C. The oven temperature program was as follows: initial temperature of 100 °C held for 1.20 min; ramp 1: 10 °C/min from 100 °C to 150 °C; hold at 160 °C; ramp 2: 23 °C/min from 280 °C held for 12 min; ramp 3: 25 °C/min to 300 °C; held for 10 min. The mass spectrometer was operated in electron impact positive-ion mode (EI+), with the ion source temperature set at 250 °C. The electron energy was 70 eV, and the filament current was 150 µA. To eliminate carryover, the fiber underwent a second thermal desorption after each chromatographic run.

Quantitative determination of BAs in beef burgers was performed using the standard addition method (SAM): 100 µL of mixed standard solutions, containing the six selected molecules at appropriate concentrations, were added to the meat sample (0.25 g), processed, and then analyzed using SPME-GC/MS as described above. Each measurement was repeated three times.

2.5. Study of PAEs Release

2.5.1. Chemicals

DBP, DEP, and DPP standards (purity > 99%) were purchased from Sigma Aldrich. Stock solutions (1 mg/mL) were prepared in sterile filtered ultrapure water (SFUW, Sigma Aldrich) with 20% (*w*/*v*) NaCl (Sigma Aldrich) and stored in glass vials at 8 °C. Working solutions were prepared daily by diluting the stock solutions with SFUW and stored at 8 °C until use [39,40].

2.5.2. SPME-GC/MS Experimental Conditions

First, 2 g of beef burger sample was transferred into a centrifuge tube, followed by the addition of an n-pentane/methanol mixture in a 5:9 (*v*/*v*) ratio (12 mL). The tube was shaken

at room temperature on a vortex mixer for 5 min, followed by centrifugation for 10 min at 1150 RCF (SBS-LZ-4000/20, Steinberg, Hamburg, Germany). The extraction process was repeated twice, and the supernatants were combined and dried by evaporation. The resulting residue was dissolved in 1.5 mL of a 20% NaCl aqueous solution and transferred into a 1.7 mL vial before being subjected to SPME-GC/MS analysis [41].

A polydimethylsiloxane/divinylbenzene fiber (PDMS/DVB, diameter 65 µm, Sigma-Aldrich) was used for the DI-SPME procedure. PAE extraction was carried out under constant stirring for 20 min at 40 °C. To prevent a possible "memory effect", the fiber was kept at 200 °C for 30 min in the GC injector after the desorption step and before each subsequent extraction.

The GC/MS system used was the same as for the BAs determination, equipped with the same column filled with helium at the same flow rate. However, the transfer line temperature was set at 220 °C, and the injector (in splitless mode for 2 min) was maintained at 270 °C [39]. The oven temperature program was as follows: 50 °C; ramp: 10 °C/min from 50 °C to 260 °C; hold at 260 °C for 3 min. The ion source temperature of the electron impact MS was set at 250 °C. The electron energy was 70 eV, and the filament current was 150 µA. The total ion current (TIC) acquisition mode with an m/z range of 40–300 was used. Analytes were detected using extracted ion chromatograms (XICs) obtained in TIC mode [39]. Throughout all experimental procedures, the use of plastic objects of any kind (tips, containers, etc.) was always avoided.

For the quantitative evaluation of PAEs in beef burgers using the SAM, 100 µL of mixed standard solutions at known PAE concentration levels were added to 2.0 g of the meat sample, which was then extracted and analyzed as described above. Each measurement was repeated in triplicate.

2.6. Microbiological Tests

The microbiological activity of chitosan-based and commercial PE films for food packaging was tested against Aspergillus Niger (NRR3112, from the NRRL collection). To produce spores, molds were initially grown on potato dextrose agar (PDA; Sigma Aldrich) Petri dishes for 5 days at 30 °C. Once grown successfully, a stock suspension of spores was prepared by flooding the PDA plates with sterile MilliQ water containing 0.01% (v/v) Tween 80 (Sigma Aldrich). Spores were then gently scraped from the surface of the newly formed mycelia using a sterile loop. To ensure maximum homogeneity, the stock spore suspension was thoroughly mixed [42].

To determine the concentration of spores in the solution, an optical microscope (Ernst Leitz GMBH Wetzlar-HM LUX, Wetzlar, Germany) was utilized with the assistance of an improved Neubauer Brightline hemocytometer (Sigma Aldrich). The stock suspension was diluted with water to obtain 1 mL of working solutions containing 100, 500, 5000, and 10,000 spores, respectively. These solutions were then inoculated onto fresh PDA plates at room temperature. Selected packaging films were placed on the inoculated plates to cover the entire surface. The plates were subsequently air-dried at room temperature for 1 h and 30 min, followed by incubation at 25 °C in the dark for 24, 48, 72 h, 5, and 10 days. After each incubation period, the spore count was determined as described above.

The inhibitory effect of the different types of coatings was calculated with reference to a plate processed in the same manner but without any polymeric covering (RP) using the following formula:

$$\text{Inhibition\%} = [(\text{SP} - \text{SF})/\text{SP}] \times 100 \quad (1)$$

where

- SP is the number of spores counted on the RP.
- SF is the number of spores counted on the inoculated plates covered with chitosan-based or PE film.

Each test was performed in triplicate.

3. Results and Discussion

3.1. Effect of the Packaging on Meat Quality after Storage: Study of the BAs Evolution

According to Leceta et al. [33], measurements of T and WVTR confirmed that the chitosan-based films, regardless of chitosan's molecular weight, exhibited transparency (T~88% in the range of 405–780 nm) and demonstrated a water vapor barrier property like that of the PE film (WVTR~8 g/m^2/day). This characteristic assists in maintaining the quality of the stored foodstuff. Figure 1, for instance, displays an example of an LMW chitosan-based film. The film appeared light yellow, possibly due to the addition of GLY [33].

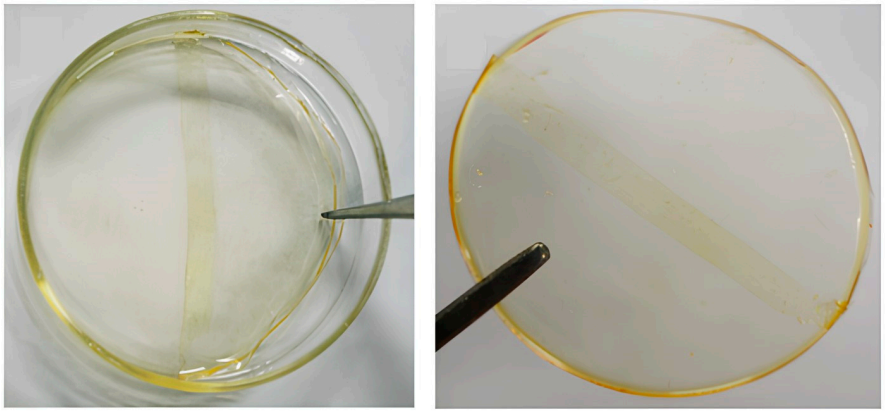

Figure 1. LMW chitosan-based film.

To investigate the possible use of the developed films in preserving meat products under domestic conditions after opening their sales packaging, the production of six BAs by "ready-to-cook" beef burgers, wrapped in chitosan-based and commercial PE films designed for food use (CTR film), was monitored.

Figure 2 shows the XIC of a beef burger sample at t0 (A) and after the addition of BAs standards (B). The IS concentration was 1 µg/mL.

Only isobutyl amine and tyramine, both at the limit of quantification (LOQ) level (47.8 and 6.2 µg/g, respectively) [8], were observed in the meat sample immediately after opening the sales packaging.

Figure 3A presents the total BA concentration levels in beef burgers wrapped in the different packaging materials, adjusted for weight loss (approximately 5% for all samples, as expected from the literature [8,43]), at various storage times at 8 °C.

After storage, the total BAs levels in each beef burger sample increased. Isobutyl amine (Figure 3B), putrescine (Figure 3C), and tyramine (Figure 3D) were found in all the samples, while butylamine, cadaverine, and isopentyl amine were predominantly detected in the PE-stored meat. The chitosan-based films, particularly those with a LMW, appeared to better preserve meat (*t*-test, $p < 0.05$). These results suggest that the MW of the chitosan used may have an impact on its antimicrobial activity. Indeed, studies by Liu et al. (2006) [44] and Cruz-Romero et al. (2013) [45] indicated that LMW chitosan exhibited higher antimicrobial activity than HMW chitosan, likely due to its ability to disrupt bacterial cell metabolism [46], penetrate the cell wall, and bind with DNA, thereby inhibiting mRNA and DNA transcription [47].

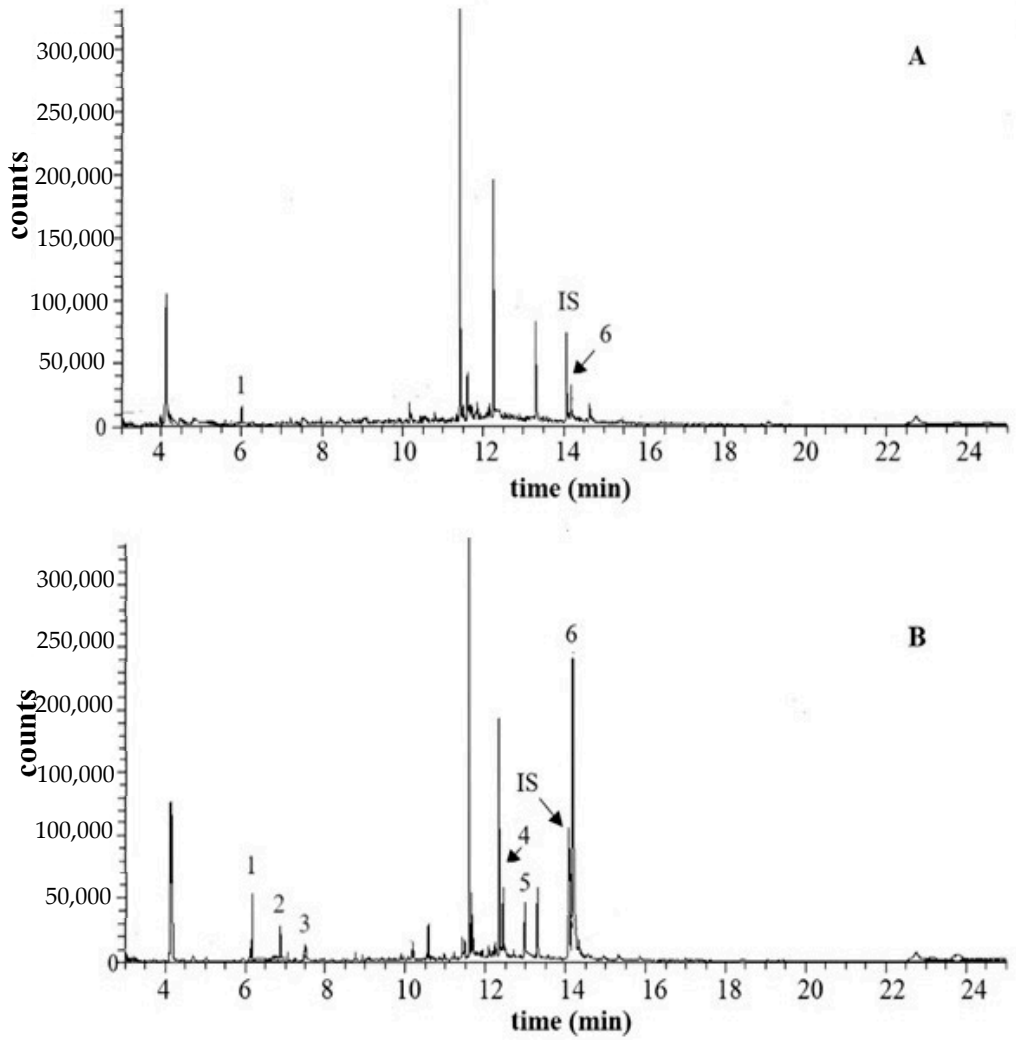

Figure 2. XICs of a beef burger sample at t0 (**A**) and after the addition of BAs standards (**B**). The extracted ions were 84, 107, 112, 118, 120, 130, 132, and 170 m/z. The concentration levels in the mixed solution were isobutyl amine (1): 250 μg/g; butylamine (2) and isopentyl amine (3): 20 μg/g; putrescine (4): 100 μg/g; cadaverine (5): 50 μg/g; and tyramine (6): 70 μg/g.

pH measurements, moreover, showed that the highest values were registered for beef burgers wrapped in the PE film for 9 days (8.15 ± 0.05). In contrast, the LMW and HMW chitosan-based films exhibited lower and similar pH values (6.76 ± 0.07 and 7.01 ± 0.5, respectively), suggesting a possible correlation with the increase in BAs concentration [8]. For beef meat, upon opening the sales packaging, a pH value of 5.72 ± 0.03 was measured (t0).

3.2. Study of Packaging's PAEs Release

The study of the possible release of four PAEs not intentionally added in stored meat products was conducted to evaluate the risks to human health deriving from the eventual use of chitosan-based packaging. Also, in this case, a commercial PE film designed for food use was considered a reference.

Figure 4 shows an XIC of a beef burger sample at t0. DEP, DPP, and DBP contaminated the beef burger at concentration levels of 0.197 ± 0.004, 0.180 ± 0.006 (LOQ level), and 0.030 ± 0.001 (LOD level) mg/Kg, respectively. As the original packaging was free of synthetic additives and PVC, as reported by the producer, it is correct to hypothesize that these molecules were present as NIASs.

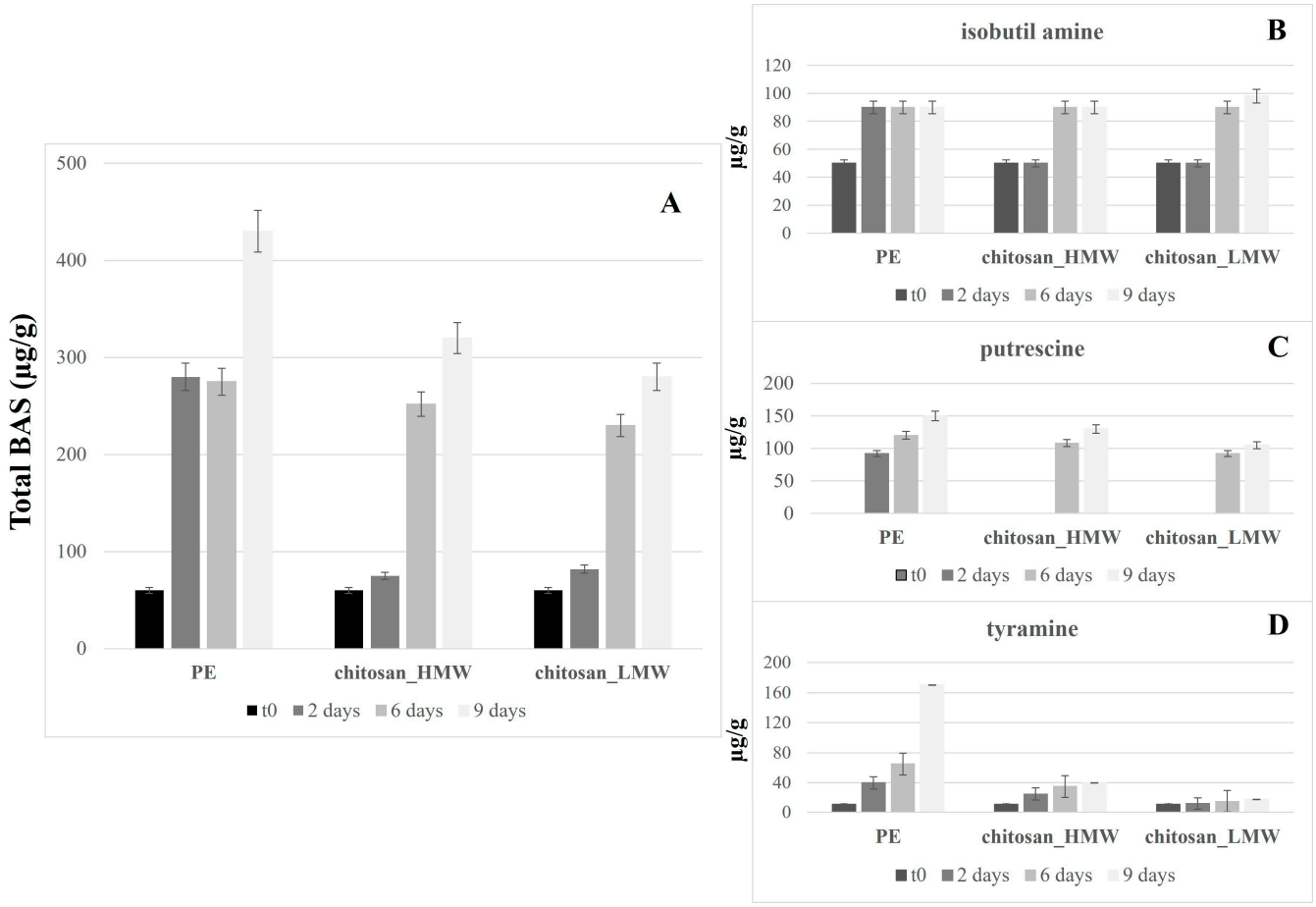

Figure 3. Total BAs (**A**), isobutyl amine (**B**), putrescine (**C**), and tyramine (**D**) concentration levels in beef burger samples wrapped in commercial PE- and chitosan-based films (LMW and HMW) at different storage times at 8 °C.

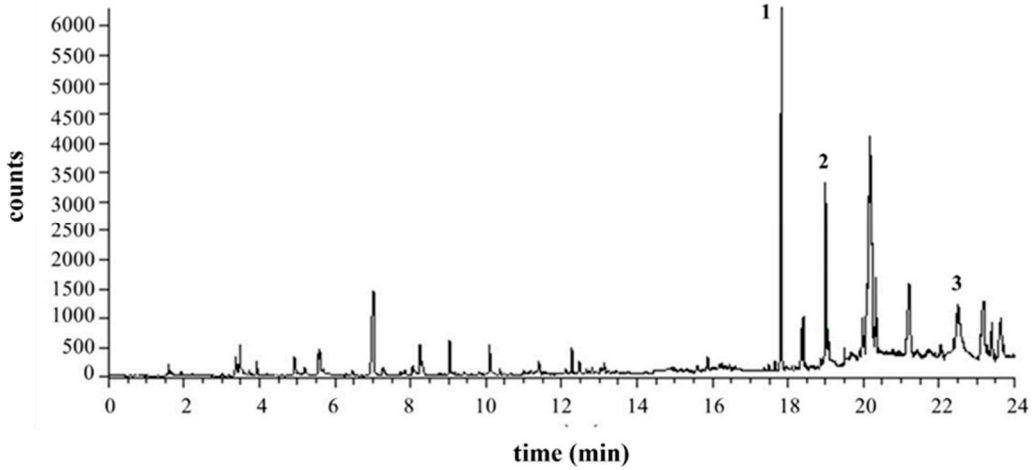

Figure 4. XIC of beef burger sample at t0. The extracted ions were 104, 149, 167, 177, 205, and 223 *m*/*z*. The peaks correspond to DEP (1), DPP (2), and DBP (3).

During the storage time, the DEP, DPP, and DBP amounts increased in the meat wrapped in the PE film. No significant variation in PAE concentration was observed for the samples wrapped in both chitosan-based packaging (*t*-test, $p < 0.5$), as shown in Figure 5, which reports the total PAE migration levels released by the three tested coatings in beef meat (mg/Kg) at different exposure times at 8 °C, estimated using the developed SPME–

GC/MS protocol [39]. The total PAE concentration level was always below 60 mg/Kg, in legal compliance [13].

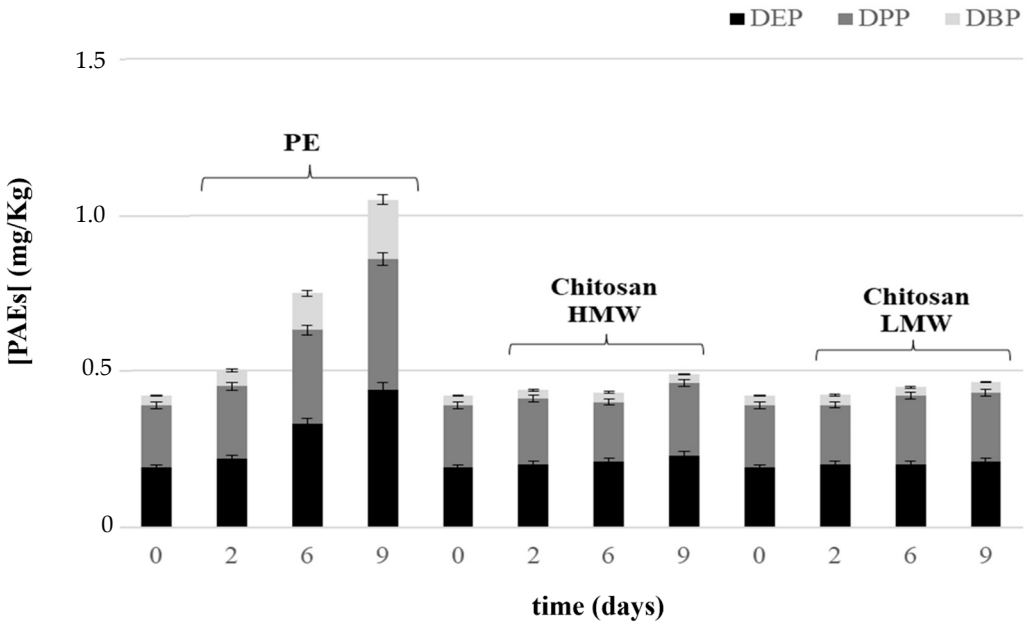

Figure 5. Total PAE migration levels in beef burgers wrapped in commercial PE- and LMW and HMW chitosan-based films after different storage times at 8 °C.

DBP is widely employed as an additive by the plastic industry, and due to its high polarity and LMW, it easily migrates from packaging to food [48]. Even if (FCM No. 157) DBP is one of the five phthalates allowed in plastic food contact materials [49], it has been listed by the European Commission on endocrine disruption and all current regulations as a priority substance [50]. Consequently, it cannot be used in cosmetics and personal care products [51–53]. For this analyte, EU Regulation No. 10/2011 [13] indicates a maximum level in the range of 0.05–0.1% (*w*/*w*) in food packaging and sets a limit of ≤0.3 mg/kg for migration levels from packaging to food. This contamination level was never reached even after the longest selected storage time for meat wrapped in each of the studied packaging types.

3.3. Microbiological Test

The evaluation of the antimicrobial activity against Aspergillus Niger NRR3112 completes the characterization of the developed chitosan-based films. This fungus, as previously highlighted, was chosen because it is often used by the food industry as a preservative.

Figure 6 shows, as an example, two Petri dishes containing the culture medium and 1000 spores of Aspergillus Niger, covered and uncovered with the LMW chitosan-based film, after 10 days of contact in the dark at 25 °C.

The microbiological tests showed that both the chitosan-based films, irrespective of their MW, possess, after 10 days of contact in the dark at 25 °C, complete antimicrobial activity against Aspergillus Niger, as does commercial PE. The prepared chitosan-based films, especially those at LMW, as well as the PE films, exhibited good water and oxygen barrier properties, which could cause a significant decrease in transpired moisture and oxygen exchange strictly associated with the antifungal activity of aerobic strains [33]. Moreover, it is possible to hypothesize the permeation of chitosan into the cells because of its action on the cellular surface [54,55]. The interaction between chitosan and Aspergillus Niger cells is mainly electrostatic [56–58]. The positive charge of the protonated amino group of chitosan interacts with the negatively charged molecules on the surface of the fungal cells. The permeabilization of the cell surface causes the leakage of intracellular substances, which eventually leads to the death of Aspergillus Niger [50,54,57,59]. Some

research results show that chitosan can also affect DNA expression by binding with nucleic acid [60–62].

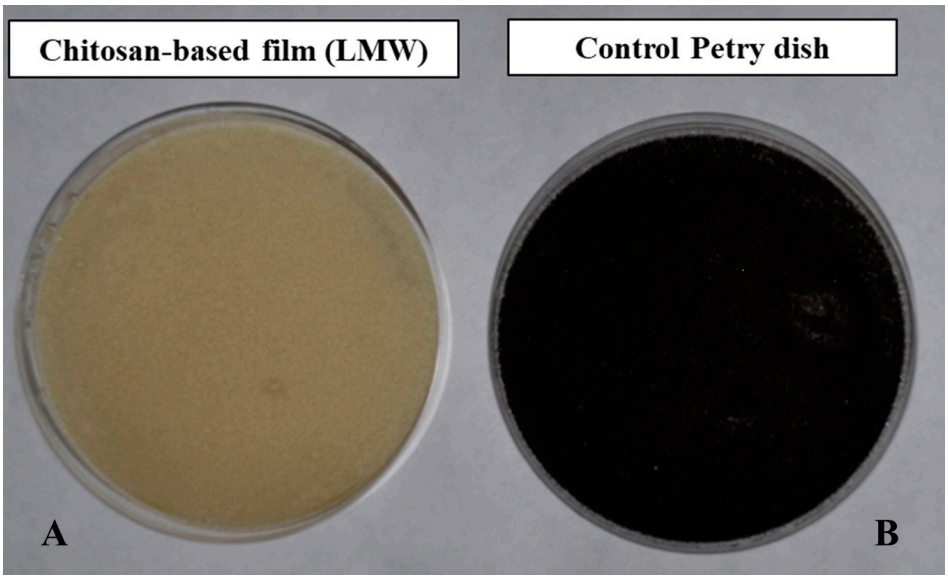

Figure 6. Petri dishes containing the culture medium and 1000 spores of Aspergillus Niger covered (**A**) and uncovered (**B**) with the LMW chitosan-based film after 10 days of contact in the dark at 25 °C.

This inhibitory effect, negative for beneficial Aspergillus Niger, could be useful to preserve meat and its derivatives from the proliferation of harmful spores responsible for producing mycotoxins (i.e., aflatoxins, ochratoxin A, etc.) [63].

4. Conclusions

A bioactive and biodegradable film based on chitosan, at both low and high MW, was presented as a potential alternative packaging material to commercial polymers (e.g., PE) for the domestic preservation of "ready-to-cook" beef burgers once the sales packaging has been opened.

A validated SPME-GC/MS protocol for the evaluation of BAs, which are indicators of putrefactive processes, enabled us to conclude that both proposed films, particularly the one based on LMW chitosan, could enhance the shelf-life of meat. The meat was stored in a home refrigerator at 8 °C, showing better results than commercial PE (CF).

Additionally, SPME-GC/MS measurements confirmed that all the considered packaging materials (chitosan-based films and commercial PE) contained PAEs as contaminants, but these molecules were released into the beef meat at levels always below the legal limit for total migration.

Finally, microbiological tests demonstrated that all the analyzed films were effective in completely inhibiting the growth of Aspergillus Niger spores.

Author Contributions: N.D.V. and A.M.A. conceived the original idea. A.M.A. prepared the chitosan-based films. N.D.V., G.M. and A.M.A. carried out the SPME–GC/MS analysis, also validating the indicated analytical methods and processing all the experimental data. The manuscript was written by N.D.V., A.M.A. and C.Z. C.Z. also provided the financial support. All authors have read and agreed to the published version of the manuscript.

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Research and innovation network on food and nutrition Sustainability, Safety and Security—Working ON Foods".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

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

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