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# Application of chitosan-sunflower oil edible films to pork meat hamburgers

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**Abstract**

Edible films were prepared by combining high molecular weight chitosan with sunflower oil. Films were obtained by casting and were applied to the surface of pork meat hamburgers. Chitosan-based films increased the metmyoglobin (MtMb) content of coated hamburgers during cold storage, especially when using lactic acid as a solvent. The incorporation of sunflower oil to the chitosan matrix led to a reduction in MtMb content of hamburgers as compared to samples coated with pure chitosan films. Chitosan films led to a reduction in the microbial counts of samples during storage. However, it is important to modulate the oxygen permeability of films in order to avoid undesirable effects (MtMb formation).



*Keywords:* chitosan; film; coating; microbial quality; pork meat

## 1. Introduction

Chitosan is a cationic biopolymer, which shows antioxidant and antimicrobial properties and can be used as a matrix to develop edible films with different food applications [1]. Chitosan-based films tend to be brittle and show high water vapour permeability. The incorporation of lipid compounds, such as sunflower oil could improve mechanical and barrier properties of chitosan films to adapt them to a



curs finally at the surface. The use of biodegradable films containing antimicrobial agents could be an alternative to extend meat shelf-life, by maintaining high concentrations of antibacterial ingredients that can also be extended throughout transport and storage period.

The aim of this work is to assess the feasibility of using chitosan-sunflower oil edible films to extend the shelf-life of cold-stored pork meat hamburgers.

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**Nomenclature**

| | |
|---|---|
| FFD | film-forming dispersions |
| CH | chitosan |
| A | acetic acid |
| L | lactic acid |
| S | sunflower oil |
| RH | relative humidity |
| PET | polyethylene |
| TBARS | thiobarbituric acid reactive substances |
| MtMb | metmyoglobin |
| WVP | water vapour permeability |

## 2. Materials and Methods

### *2.1. Preparation and characterization of chitosan-based films*

High molecular weight chitosan (CH) (Batch 12913CJ, Sigma-Aldrich, USA), was dispersed at 1 wt% in an acetic acid (A) or lactic acid solution (L) (Panreac, Barcelona, Spain) at 1 wt%. To prepare the composite films, sunflower oil (Koipesol, Madrid, Spain) was added to the chitosan solution at 1 wt%. This mixture was homogenized by means of a rotor-stator (DI25, Yellow Line, IKA®, Germany) at 21500 rpm for 4 minutes. Afterwards, it was submitted to high-pressure homogenization at 165 MPa in a single pass by means of a Microfludizer® M110-P processor (Microfludics, Newton, USA). Films were obtained by casting and drying at room temperature and 60% RH. Surface density was 56 mg/cm$^2$. Films were peeled off from the casting plates and conditioned at 58%RH and 5ºC for one week. Water vapour permeability and gloss of the films were measured in triplicate following the methodology described by Vargas et al. [3]. Four kind of films (CH_A, CH_A:S, CH_L, CH_L:S) containing CH, acetic acid (A) or lactic acid (L), and sunflower oil (S) were prepared.

### *2.2. Hamburger manufacture and application of films*

Pork meat was obtained from a local supermarket. The meat was ground by using a mincer (Severin Elektrogeräte GmbH, Sundern, Germany) and was moulded in Petri dishes to obtain the hamburgers. The surface of both sides of the hamburgers was coated with the films. Non-coated and coated samples were placed in PET trays (Cubil, Barcelona, Spain) and were stored at 4 ºC.

### *2.3. Analyses*

Thiobarbithuric acid reactive substances (TBARS) and metmyoglobin (MtMb) assays were performed in triplicate following the same procedure described by Fernández-López et al. [4]. All reagents used to perform TBA and MtMb analyses were supplied by Sigma (Sigma-Aldrich, USA).

To perform microbiological analyses, a 10 g aliquot of each sample was aseptically obtained and homogenized in a Stomacher with 90 mL of sterile buffered peptone water. Aliquots were serially diluted in buffered peptone water and plated out following standard methodologies. Total viable counts were determined in Plate Count Agar plates incubated at 37ºC for 48ºC. Coliform counts were determinate

using Violet Red Bile Agar plates incubated at 37ºC for 24h. All microbial analyses were performed in triplicate and all culture media were from Scharlau (Scharlab S.L, Barcelona, Spain).

## 3. Results and Discussion

Water vapour permeability values and gloss of the stand-alone films are shown in Table 1.

Table 1. Water vapour permeability (5ºC, 100/58% RH gradient) and gloss of chitosan-sunflower oil films. Mean values and standard deviation (in brackets)

| Film | WVP x 1011 ($g \cdot Pa^{-1} \cdot s^{-1} \cdot m$) | Gloss 60º | Gloss 85º |
|---|---|---|---|
| CH_A | 345 (240) | 22 (16) | 20 (12) |
| CH_A:S | 60 (36) | 55 (22) | 33 (26) |
| CH_L | 280 (63) | 14 (4) | 18 (9) |
| CH_L:S | 352 (321) | 21 (11) | 22 (16) |

The different solvent used to prepare the films did not have a significant effect on the water vapour permeability of films. The incorporation of sunflower oil to chitosan matrix led to a reduction in the water vapour permeability of CH_A films, in agreement with the reported effect of incorporating unsaturated fatty acids such as oleic acid into chitosan films prepared with acetic acid [3].

Acetic acid yielded glossier films. For both solvents, the incorporation of sunflower oil into the chitosan matrix led to an increase in the gloss. This is in agreement with the reported effect of incorporating oleic acid in chitosan edible films [3]. The increase in gloss, in line with addition of sunflower oil, could be explained by the coalescence and creaming of the oil droplets during film drying. This led to a decrease in surface roughness and an increase in specular reflectance in the air-film interface, since the oil can fill the small surface voids generated during the film formation. In general, the smoother the surface the higher the gloss [5].

The average MtMb content at the beginning of the storage was 39%. MtMb content of all samples increased steadily during the whole period, which is in agreement with the observed trend in cold-stored fresh pork meat samples [6]. Moreover MtMb content was significantly higher in coated samples ($p<0.05$) after 4 days of storage. Changes in MtMb content of coated samples with reference to non coated ones as a function of coating formulation are shown in Figure 1.

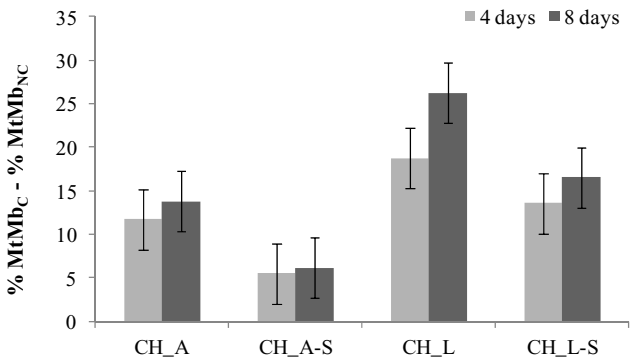


Fig. 1. Changes in metmyoglobin content of coated samples (C) with reference to non-coated (NC) hamburgers during storage at 5ºC. Mean values and 95% LSD intervals

Chitosan-based films promoted an increase in the MtMb content of hamburgers during storage, especially when lactic acid was used to prepare the films. This can be explained by the low oxygen permeability of chitosan films, which is in the range of high density polyethylene films [2]. Since the formation of MtMb has been related with low oxygen atmospheres [7], the increase in MtMb content detected in coated hamburgers, with regard non-coated ones, could be explained by the low oxygen pressure at the surface of the samples due to chitosan film. The incorporation of sunflower oil to the chitosan matrix led to a reduction in MtMb content of hamburgers as compared to samples coated with pure chitosan films. This can be explained by a change in oxygen permeability of films as a consequence of the incorporation of sunflower oil. In these cases the liquid lipid phase contributes to increase the oxygen diffusion through the film.

Average TBARS values at the beginning of storage were 3.7 mg malonaldehide/kg sample and did not showed significant changes during the whole storage period (data not shown). The analyses of TBA were limited by the microbial decay detected in non-coated samples at the end of storage.

Total viable and coliform counts of samples coated with chitosan-based films prepared with lactic acid or acetic acid are shown in figure 2 and figure 3, respectively.

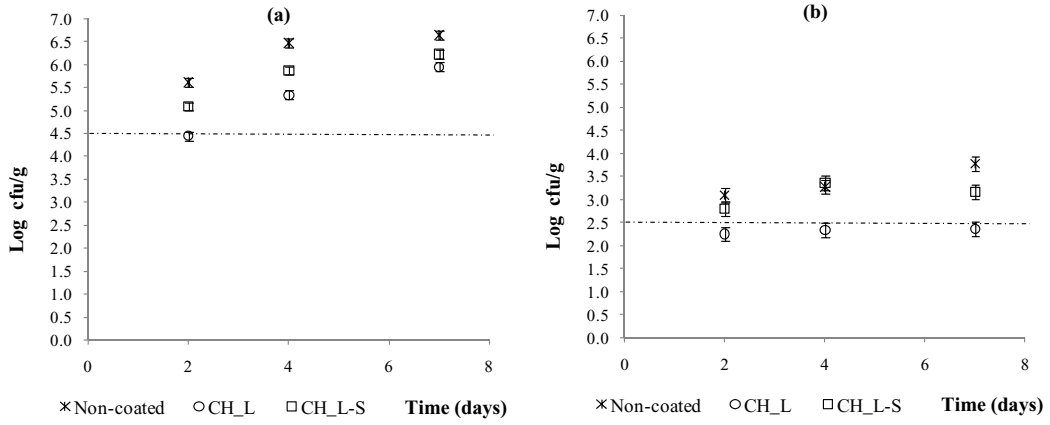


Fig. 2. Effect of chitosan-based films prepared with lactic acid on total viable (a) and coliform counts (b) of hamburgers stored at 5ºC. Mean values and 95% LSD intervals. Dashed line indicates initial count (t = 0)

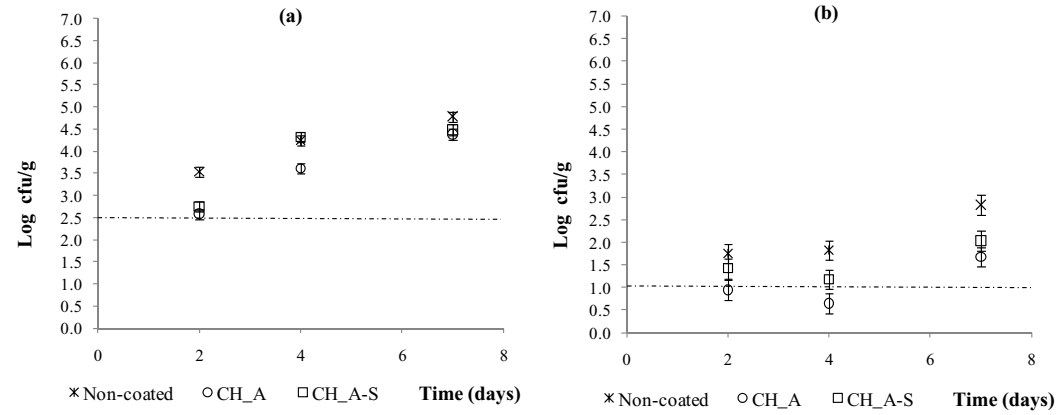


Fig. 3. Effect of chitosan-based films prepared with acetic acid on total viable (a) and coliform counts (b) of hamburgers stored at 5ºC. Dashed line indicates initial count (t = 0)

Chitosan-based films led to a reduction in the microbial counts of pork meat hamburgers during storage. This reduction was more marked in terms of total coliform counts (Figure 2b and 3b), where pure chitosan coatings shown, in some sense, a bactericide effect. In fact, pure chitosan films have been proved to be effective against Escherichia coli in studies performed with a model food system [8].

No significant differences were detected between samples coated with chitosan films prepared with acetic acid and lactic acid in terms of antimicrobial efficacy.

The addition of sunflower oil led to a reduction in the antibacterial effect of pure chitosan coatings against coliform microorganisms. This reduction was significant for films prepared with lactic acid ($p<0.05$). Since the films were prepared maintaining a constant surface solid density, the decrease in the antibacterial activity due to the incorporation of sunflower oil can be attributed to the dilution effect of CH, thus being less available for microorganisms.

## 4. Conclusion

The addition of sunflower oil to chitosan-based films yielded glossier films and led to an improvement in the water vapour barrier properties when acetic acid was used as a solvent to prepare the films.

The use of chitosan-based edible films could be a promising technique to preserve the microbial quality of pork meat hamburgers. However, it is important to modulate the oxygen permeability of films in order to avoid the undesirable effects (MtMb formation), which are promoted by the lower oxygen partial pressure in the surface of the coated hamburgers.

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