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ORIGINAL ARTICLE

Effect of chitosan addition on the inhibition of lipid oxidation in ground pork

Efecto de la adición de quitosana en la inhibición de la oxidación lipídica en carne de cerdo molida

Jorge Cruz[1] • **Mario A. García**[2] • **Nilia de la Paz**[3]

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Abstract The objective of this study was to evaluate the effect of UV irradiation on chitosan's antioxidant activity using the ABTS assay and its inhibition of lipid oxidation in ground pork through the TBA assay. Chitosan was obtained from lobster chitin (*Panulirus argus*) through a thermo-alkaline N-deacetylation process. Six treatments were applied at different times (5, 15, and 30 min) and two wavelengths (254 and 365 nm). A sample of non-irradiated chitosan was used as a comparison standard. The chromatic coordinates of the chitosan solutions were also determined. UV irradiation of chitosan did not significantly vary (p>0.05), neither in the chromatic coordinates nor in the antioxidant activity of its 1% (w/v) solutions under the tested conditions. Adding the chitosan solution in 1% (v/v) lactic acid at a ratio of 2.5 mL per 50 g of ground pork reduced lipid oxidation from 0.23 to 0.14 mg MDA/kg after 24 hours at room temperature.

Keywords chitosan, irradiation, antioxidant activity, lipid oxidation, ground pork.

Resumen El objetivo del presente trabajo fue evaluar el efecto de la irradiación UV de la quitosana en su actividad antioxidante mediante el ensayo ABTS e inhibición de la oxidación lipídica en carne de cerdo molida mediante el ensayo TBA. Se utilizó quitosana obtenida de la quitina de langosta (*Panulirus argus*), a través de un proceso de N-desacetilación termo-alcalina. Se aplicaron seis tratamientos a diferentes tiempos (5; 15 y 30 min) y dos longitudes de onda (254 y 365 nm). Como patrón de comparación, se empleó una muestra de quitosana no irradiada. También se determinaron las coordenadas cromáticas de disoluciones de las quitosanas. La irradiación UV de la quitosana no varió significativamente (p>0,05), ni las coordenadas cromáticas, ni la actividad antioxidante de sus disoluciones al 1 % (m/v) en las condiciones ensayadas. La adición de la disolución de quitosana en ácido láctico al 1 % (v/v) a razón de 2,5 mL por 50 g de carne de cerdo molida, redujo su oxidación lipídica desde 0,23 hasta 0,14 mg MDA/kg a las 24 h a temperatura ambiente.

Palabras clave quitosana, irradiación, actividad antioxidante, oxidación lipídica, carne de cerdo molida.

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✉ Mario A. García
mario.garcia@sangregorio.edu.ec

1 Instituto de Farmacia y Alimentos, Universidad de La Habana, Cuba.
2 Universidad Técnica de Manabí, Ecuador.
3 Centro de Investigación y Desarrollo de Medicamentos, La Habana, Cuba.

PUBLIS EDITORIAL

Introduction

Food safety remains a critical public health issue due to the constant outbreaks of foodborne diseases. In recent years, an increase in food poisoning, especially in animal-origin foods, has been observed. This is attributed, in part, to the growth of global trade, changes in food production, and new lifestyles that have altered food consumption patterns.

According to the FAO and WHO, food contamination causes significant economic losses in the food industry. Therefore, various chemical, physical, and new technological processes have been developed to extend the shelf life of foods, highlighting the use of natural antioxidants as a promising solution. In this context, chitosan has become as an effective preservative for animal-origin foods (No et al., 2007; Dutta et al., 2009). Moreover, its production from seafood industry waste, such as crustacean exoskeletons, offers an opportunity to utilize these residues, as they are a rich source of chitin.

Chitin is the second most abundant natural polymer after cellulose and is widely distributed in nature. Its high replenishment rate in the biosphere makes it an important renewable resource (Hernández et al., 2009). The main source of chitin comes from crustacean exoskeletons, such as lobsters, which contain high concentrations of this polymer. Chitosan can be obtained from chitin through a chemical N-deacetylation process. Due to its functional and physicochemical properties, chitosan has applications in various fields, including food, medicine, agriculture, cosmetics, and pharmaceuticals. Although there are methods for obtaining and characterizing it, its use remains limited by the variability in its chemical composition, degree of deacetylation, and chain size (Hernández et al., 2009).

The presence of amino groups in the structure of chitosan gives it great versatility for making modifications, such as enzyme immobilization, grafting reactions, and the creation of cross-linked films. These modifications allow the production of materials suitable for applications in biotechnology, food, and medicine.

One of the key properties of chitosan in the food industry is its antioxidant capacity, primarily attributed to its efficiency in chelating metal ions, and preventing lipid oxidation (Rhazi et al., 2002; Guibal, 2004). However, the exact mechanism of its antioxidant action is still debated.

Previous studies (Youn et al., 2008) have demonstrated that high-viscosity decolorized chitosan can be obtained through UV irradiation without bleaching agents, improving the product's sensory characteristics and facilitating its commercialization. Additionally, UV irradiation treatment has shown promise as a large-scale chitosan production technology, reducing energy consumption by effectively decolorizing chitosan. However, further research is needed on the effects of UV irradiation dosage on the properties of chitosan obtained from lobster chitin (*Panulirus argus*). The objective of this research was to evaluate the effect of UV irradiation on chitosan and its impact on antioxidant activity and the inhibition of lipid oxidation in ground pork.

Materials and methods

Chitosan from common lobster (*P. argus*) was used, and obtained on a pilot scale at the Natural and Synthetic Products Production Plant of the Center for Drug Research and Development in Havana, Cuba (de la Paz et al., 2012). Chitosan is insoluble in water due to its high molecular weight; in this study, water was used as a dispersing medium at a ratio of 16 mL/g of chitosan with constant stirring at 120 rpm to ensure uniform treatment of the polymer during its UV irradiation.

Six treatments (Table 1) were sequentially applied at room temperature (28 ± 0.5 ºC) with different time intervals (5, 15, and 30 min) and wavelengths (254 and 365 nm) using a low-intensity UV lamp (YL, Mod. WD-9403E, Beijing Liuyi Instrument Factory, China). Immediately after UV irradiation, the chitosan samples were filtered and dried at 60 ºC for 4 hours. They were then hermetically sealed until used for evaluating the antioxidant capacity and chromatic coordinates of their 1% (w/v) solutions.

Table 1. UV irradiation treatments of chitosan

Treatment	Wavelength (nm)	Time (min)
1*	-	-
2		5
3	254	15
4		30
5		5
6	365	15
7		30

* Control treatment.

Chitosan solutions at 1.0% (w/v) were prepared in a 1% (v/v) lactic acid solution with stirring using a magnetic stirrer for 2 hours. Previously, Tween 80 was added at 0.1% (v/v) to the 1% (v/v) lactic acid solution. The total antioxidant capacity was evaluated according to the methodology proposed by Re et al. (1999) and Chien et al. (2007), with some modifications. The color determination of the chitosan solutions was performed using a spectrophotometric method, according to the methodology described by Casariego (2009). A spectrophotometer (Shimadzu UV-2401PC UV-VIS, Japan) was used to obtain the transmittance spectrum in the visible region between 400 and 700 nm.

The meat was taken from the leg and nerve region of the same animal. All visible fat was then removed. The meat was

ground using a food processor (Sumeet, Mumbai, India) and then divided into two groups: a control group and another group to which the chitosan solution in 1% (v/v) lactic acid was added at a ratio of 2.5 mL per 50 g of ground meat. The mixture was thoroughly combined and packed in sealed plastic tubes.

The antioxidant capacity was determined using the TBA method, following the methodology proposed by Vyncke (1975) with modifications. The results were expressed as malondialdehyde in mg/kg of sample.

A two-way analysis of variance was performed using the Statistics software (version 7, 2004, StatSoft. Inc., Tulsa, USA) and Duncan's multiple range test to compare differences between the evaluated samples for $p \leq 0.05$.

Results and discussion

Table 2 shows the behavior of the antioxidant capacity of chitosan DFC (1% w/v) with different UV treatments. As can be seen, the wavelength and application time did not influence (p>0.05) the antioxidant capacity of the solutions, with values ranging from 4.93 to 5.48 mg Trolox/100 mL.

Table 2. Influence of UV irradiation of chitosan on the antioxidant capacity of 1% (w/v) solutions

Treatment	Antioxidant capacity (mg Trolox/100 mL)
1	4.93 (1.1) a
2	4.96 (1.0) a
3	5.01 (0.8) a
4	5.38 (1.8) a
5	5.48 (2.1) a
6	5.09 (1.3) a
7	5.12 (1.5) a

Mean (Standard deviation); n= 3.

Different letters indicate significant differences (p≤0.05) according to Duncan's multiple range test.

UV irradiation can induce the formation of new polar groups in the chitosan molecule through photooxidation, generating radicals that, in the presence of oxygen, can give rise to carbonyl, hydroxyl, and hydroperoxide groups (Rabek, 1995). In particular, $OH^{\bullet}$ radicals derived from irradiated chitosan are highly reactive and can interact with macromolecules, producing new radicals and macroradicals (Rabek, 1995). Given that chitosan contains numerous OH groups, the formation of a large number of $OH^{\bullet}$ radicals and macroradicals is possible, which could generate cross-linked structures and affect the polymer's antioxidant capacity.

It could be expected that UV irradiation increases chitosan's antioxidant capacity due to photochemical changes, such as polymer chain scission (Sionkowska et al., 2006), resulting in the cleavage of the acetal bond (C1 and C4), which would form new active centers capable of reacting with highly reactive species like free radicals (Figure 1). Additionally, irradiation could cause amide bond cleavage, leading to partial deacetylation of the molecule (Rashid et al., 2012). The degree of deacetylation of chitosan, determined by FTIR-ATR, slightly decreases after UV treatment (Mucha & Pawlak, 2002).

Various studies have evaluated the antioxidant activity of aqueous chitosan solutions, reporting its ability to scavenge hydroxyl radicals (Xie et al., 2001) and metals (Xue et al., 1998). The antioxidant activity of chitosan can be explained through several mechanisms. One of them is its ability to neutralize free radicals, where nitrogen at C-2 of the polymer plays a fundamental role. It has been suggested (Xie et al., 2001) that this ability is related to the reaction of free radicals with hydrogen ions from ammonium ions (NH3+) present in stable chitosan molecules (Yen et al., 2008).

Researchers like Chien et al. (2007) examined the antioxidant activity of three types of chitosan with different molecular weights, reporting that chitosan with 12 kDa had the highest antioxidant activity, with 2.15 µmol of Trolox equivalents, compared to 1.46 µmol and 0.89 µmol for chitosans with 95 kDa and 318 kDa, respectively. The antioxidant capacity of natural compounds manifests both in the termination stage of the free radical formation mechanism and in their reducing power, which reflects the ability to transfer electrons, being a significant indicator of the potential antioxidant activity of a compound (Meir et al., 1995).

Kim & Thomas (2007) noted that the ability of chitosan to scavenge free radicals depends on the polymer's concentration and molecular weight. However, Sweetie et al. (2008) indicated that chitosan has limited antioxidant capacity, obtaining low values in the DPPH assay. Although the nitrogen atom in chitosan has a pair of unshared electrons that could be donated, in aqueous solutions, the -NH2 groups are mostly protonated, which hinders this electron donation. Additionally, chitosan lacks a readily donatable hydrogen atom, limiting its capacity as an antioxidant (Schreiber et al., 2013). In contrast, phenolic compounds, classified as primary antioxidants, act by donating a hydrogen atom or an electron, and the resulting phenoxyl radicals are stabilized through electron delocalization in the aromatic ring (Eskin & Przybylski, 2000; Leopoldini et al., 2011).

Generally, a trend has been observed in which the antioxidant capacity of chitosan increases as its molecular weight decreases, as reported in previous studies. However, it is important to consider several factors that can influence the results, such as the polymer concentration, the ratios between the reagent and the sample, and differences between

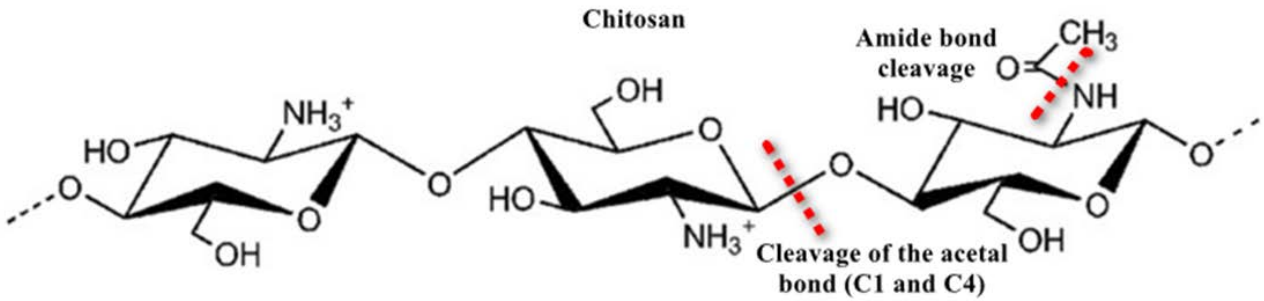

Figure 1. Bond breakage in the chitosan molecule.

the chitosans used in each investigation, which limits direct comparisons of the results.

According to Frankel & Meyer (2000), multiple factors influence the effectiveness of antioxidants in heterogeneous and complex systems such as foods and biological systems. These factors include the properties of the lipid and aqueous phases of the antioxidant, the oxidation conditions, and the physical state of the substrate susceptible to oxidation. Since the influence of all these parameters cannot be evaluated with a single test method, the cited studies have employed different techniques to determine the antioxidant capacity of chitosan. All the studies agree on demonstrating chitosan's protective effect against oxidation reactions.

The different assays used to estimate antioxidant capacity allow either for evaluating whether a compound can act as an antioxidant in one or more ways, *in vivo* or in food matrices. They can also indicate that antioxidant action is possible when a compound shows in vitro protection at concentrations relevant to foods or biological systems. However, an antioxidant that works in vitro will not necessarily be effective in vivo or in foods, as it might not be absorbed, might not reach the appropriate site of action, or might be rapidly metabolized into inactive products (Halliwell, 2002). Therefore, it is crucial to evaluate the effect of chitosan coatings as an active packaging method to inhibit lipid oxidation in foods.

Finally, the L*, a*, and b* values of the chitosan solutions presented in Table 3 show that neither the wavelength nor the exposure time significantly influenced (p>0.05) the chromatic coordinates of the solutions, with luminosity (L*) values ranging between 67.39 and 70.04.

When analyzing the values of the component a*, it was observed that there were no significant differences between each treatment. The b* value is the parameter that describes the color of the solutions, due to the yellow color of the chitosan, and this is the chromatic component that most influences the total color difference (ΔE*) between the chitosans. The chromaticity values (C*) showed a similar behavior to that described for the b* component. It is widely accepted that color changes are related to possible chemical and biological changes in a substance. It should be noted that not only the source of chitosan but also the extraction process influences its color (Peniche, 2006).

Youn et al. (2007) reported that the color values for chitosan dried in the sun for 4 hours were: L* = 86.53; a* = -0.98; and b* = 10.4, values that are much lower than those obtained in this study, which could be due to the factors influencing color and may also be affected by the intensity of UV light used. Among the factors that did not affect the decolorization are the chitosan/water ratios and the stirring speed, as neither of these factors significantly influenced color; they were set according to the best results obtained from previous studies (Youn et al., 2008).

Table 3. Effect of UV irradiation of chitosan on the color of the solutions

Treatment	L*	a*	b*	C*
1	70.04 (0.372) a	1.96 (0.21) a	13.11 (0.56) a	13.25 (0.42) a
2	67.83 (0.005) a	1.90 (0.04) a	12.64 (0.66) a	12.76 (0.46) a
3	68.61 (1.843) a	3.10 (1.28) a	12.64 (0.64) a	13.04 (0.22) a
4	67.39 (0.013) a	1.67 (0.06) a	13.19 (0.80) a	13.28 (0.55) a
5	70.01 (0.437) a	1.83 (0.04) a	13.77 (0.34) a	13.89 (0.24) a
6	69.26 (0.596) a	1.62 (0.21) a	12.50 (0.05) a	12.60 (0.01) a
7	68.44 (0.647) a	1.99 (0.25) a	13.27 (0.57) a	13.41 (0.42) a

Mean (Standard deviation); n = 2.

Different letters indicate significant differences (p≤0.05) by Duncan's multiple range test.

Figure 2 shows the effect of inhibiting lipid oxidation in pork with 2 mL of 1% chitosan solution, where a decrease in lipid oxidation in the chitosan-treated pork was evident 24 hours after the start of the treatment compared to the control sample (p>0.05), which maintained the highest MDA value.

The decrease in peroxide concentration is due to the biopolymer acting as a barrier against oxygen diffusion onto the meat, which slows the formation of hydroperoxide derivatives. The macromolecule also mitigates the impact of secondary oxidation; this is due to chitosan, which has amino groups capable of reacting with malondialdehyde, causing a reduction in the levels of this aldehyde to below 1 mg MDA/Kg, considerably delaying the formation of volatile compounds that may negatively affect sensory properties.

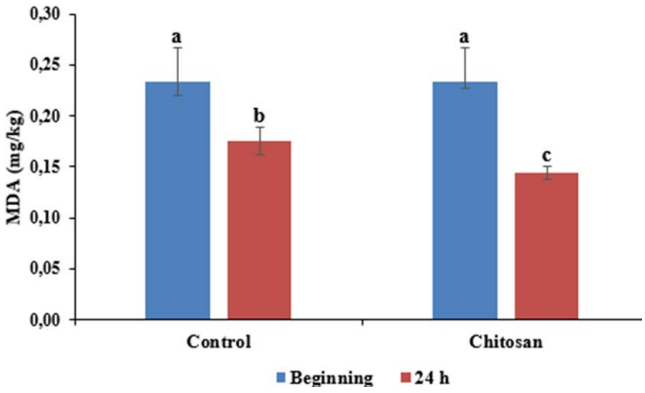

Figure 2. Inhibition of lipid oxidation by the addition of chitosan in ground pork at room temperature for 24 hours.

In other studies, Darmadji & Izumimoto (1994) observed that the addition of 1% w/v chitosan to ground beef significantly reduced the thiobarbituric acid (TBA) value compared to the control sample, demonstrating that the addition of chitosan decreases lipid oxidation in meat, resulting in a desirable effect on the stability of the red color of the product during storage. Lee et al. (2003) observed that pieces of pork immersed in chitosan solutions with molecular weights of 30 and 120 kDa at 1% (w/v) had a longer shelf life and lower lipid oxidation.

Other authors have also reported the use of chitosan as an antioxidant and concluded that this effect improves the appearance of meat, related to color, and reduces the unpleasant odor generated by the rancidity of the lipids inherent to the meat (Kanatt et al., 2008; Rao et al., 2005).

Pasanphan et al. (2010) suggested that chitosan began to be used as a natural antioxidant alternative due to the ability of oligomers from chitosan solutions to trap hydroxyl radicals through ionic reactions with the amino groups in its chemical structure.

Kanatt et al. (2004) concluded that lamb meat with irradiated chitosan increased lipid peroxidation. Ahn et al. (1998) observed that raw pork vacuum-packed with irradiated chitosan increased lipid inhibition, resulting in an extended shelf life. Many authors have studied this phenomenon, showing different results regarding the inhibition time, but all conclude that chitosan has excellent properties as an inhibitor of lipid oxidation in meats.

Conclusions

UV irradiation of chitosan did not significantly vary (p>0.05) the chromatic coordinates or the antioxidant activity of its 1% (w/v) solutions under the tested conditions. The addition of a 1% (v/v) chitosan solution in lactic acid at a rate of 2.5 mL per 50 g of ground pork reduced lipid oxidation from 0.23 to 0.14 mg MDA/kg after 24 hours at room temperature.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Jorge Cruz, Mario A. García and Nilia de la Paz: Conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, visualization, drafting the original manuscript and writing, review, and editing.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on 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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