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# Effect of molecular weight reduction by gamma irradiation on the antioxidant capacity of chitosan from lobster shells

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ABSTRACT

This study assessed the effect of molecular weight (MW) reduction by gamma irradiation on the antioxidant capacity of chitosan with potential application in the preservation of foodstuffs. Two batches of chitosan were obtained by heterogeneous chemical N-deacetylation of chitin from common lobster (*Panulirus argus*). Irradiation of chitosan was performed using a $^{60}$Co source and applying doses of 5, 10, 20 and 50 kGy with a dose rate of 10 kGy/h. Attenuated Total Reflection Fourier Transform Infrared Spectroscopy was used to identify main chemical features of chitosan. The average viscosimetric MW was determined by the viscosimetric method while the deacetylation degree by a potentiometric method. Thermogravimetric analysis and differential scanning calorimetry were conducted to evaluate the thermal degradation behavior of the chitosan samples, both under nitrogen flow. The antioxidant activity of chitosan solutions at 1% (w/v) in lactic acid at 1% (v/v) and Tween 80 at 0.1% (v/v) was evaluated through the ABTS assay and scavenging of DPPH radical by chitosan. The increase of irradiation dose with $^{60}$Co until 50 kGy decreased significantly the MW of chitosan through the scission of glycosidic bonds without affecting its functional groups, while the DD (72–75 %) did not vary ($p > 0.05$). The AC of the chitosan solutions increased with the reduction of MW of chitosan by gamma irradiation.



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## 1. Introduction

Researches related with effective natural antioxidants for food preservation has increased during the recent years. Consumer behavior has changed from the use of chemical preservatives to the demand for natural additives, especially in ready-to-eat and fresh food products. Consequently, food industry needs to find alternative methods for preservation that covering the same antimicrobial or/and antioxidant properties and compatibility with food than the chemical additives.

Many natural compounds with antioxidant capacity for extending the shelf life of foods have been studied. The use of food additives from natural source involves the isolation, purification, stabilization and its incorporation to food without adversely affecting sensory, nutritional and safety features.

Chitosan, N-deacetylated derivative from chitin, can be included in this category (Hou et al., 2012). It is widely used because of its film-forming properties, good biocompatibility, biodegradability, low cost (Sirinivasa, Ramesh, Kumar, & Tharanathan, 2004), safety (Argullo, Albertengo, Pastor, Rodríguez, & Valenzuela, 2004), and be a renewable resource. The use of chitosan as antioxidant additive had been reported in numerous researches, which had demonstrated the capacity of this polymer for interacting with free radicals through ionic interactions with its amino groups (Mahdy, El-Kalyoubi, Khalaf, & Abd, 2013). Applications as antioxidant include the preservation of strawberries (Wang & Hao, 2013), orange (Martín-Diana, Rico, Barat, & Barry-Ryan, 2009) and apple juices (Chien, Sheu, Huang, & Su, 2007), peanuts, potato chips (Schreiber, 2012), beef hamburger (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007), fermented dried sausages (Krkic et al., 2013) and mayonnaise (García, Silva, & Casariego, 2014).

Several studies *in vitro* and *in vivo* had demonstrated that de antioxidant activity of chitosan is dependent on its MW (Mahdy et al., 2013). Thus, chitosan with lower MW showed a higher antioxidant activity. Moreover, the low solubility of chitosan is related with its high MW, which affects the applications of this polymer.

Ionizing radiations such us gamma irradiation, constitute one of the most popular tools for modifying the physical and chemical properties of some polymeric materials (Choi, ParK, Ahn, Lee, & Lee, 2002). In that sense, the gamma irradiation can be used to improve its solubility (Mao et al., 2004; Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005) by decreasing the MW (Chmielewski, 2010; Ciechanska et al., 2004) by breaking the polymeric chains and thus enhance its antimicrobial and antioxidant properties (Chmielewski et al., 2007).

Although various studies have reported the application of irradiation in the modification of polymers such us chitosan and it is commercially available, the information about the relationship between the irradiation of chitosan and some of its biological properties is still limited. However, some papers informed about the influence of chitosan MW in its antioxidant (Kim & Thomas, 2007) and antimicrobial (Tikhonov et al., 2006) properties and as biostimulator for plant growing (Gryczka, Gawrońska, Migdał, Gawroński, & Chmielewski, 2008). Accordingly, the present study assessed the effect of MW reduction by gamma irradiation on the antioxidant activity of chitosan derived from lobster chitin by heterogeneous chemical N-deacetylation with potential applications in the preservation of foodstuffs.

## 2. Materials & methods

### 2.1. *Irradiation with $^{60}$Co*

Two batches of chitosan, one at lab scale (Lot I) and the other at pilot scale (Lot II), were obtained at the Drug Research and Development Center (Havana, Cuba), by heterogeneous chemical N-deacetylation of chitin from common lobster (*Panulirus argus*). Irradiation of chitosan was performed at the Center of Technological Applications and Nuclear Development (Havana, Cuba) using a $^{60}$Co source and applying doses of 5, 10, 20 and 50 kGy with a dose rate of 10 kGy/h in an irradiation facility Gamma PX-30. The distribution dose in the installation as well as the calibration of the irradiation process were verified through the Fricke dosimetrical system, while that for controlling the process were used dosimeters Red Perspex (Barrera, Otero, Rodríguez, & González, 2005). Before irradiation, the chitosan was packaged in bags of low-density polyethylene with 50 μm of thickness.

### 2.2. *Chemical characterization*

#### 2.2.1. *Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FT-IR)*

ATR-FT-IR was used to identify main chemical features of chitosan. ATR-FT-IR spectra were recorded on a Nicolet 6700 spectrophotometer in the 4000–400 $cm^{-1}$ range with a diamond ATR crystal. The spectra were recorded with a resolution of 4 $cm^{-1}$ and an accumulation of 64 scans.

#### 2.2.2. *Molecular weight (MW)*

A capillary viscometer Ubbelhode No. 2121R with a constant temperature bath controlled by recirculating water (Haake, Germany) at 25.0 ± 0.01 °C was used to determine the average viscosimetric MW. The chitosan solutions were prepared using the solvent system lactic acid at 0.1 mol/L and sodium chloride at 0.2 mol/L. The initial polymer concentration was $9.6 \times 10^{-3}$ g/mL in all cases and four dilutions ($7.68 \times 10^{-3}$, $5.76 \times 10^{-3}$, $1.92 \times 10^{-3}$ and $9.6 \times 10^{-4}$ g/mL) were prepared. The fall time of each of the polymeric solutions was measured with five replicates for determining the viscometric parameters (Table 1).

**Table 1 – Viscosimetric parameters for determining viscosimetric molecular weight.**

| Parameter | Symbology and equation |
|---|---|
| Relative viscosity | $\eta_r = \eta/\eta_0 = t/t_0$ |
| Specific viscosity | $\eta_{sp} = \eta_r - 1 = (\eta - \eta_0)/\eta_0 \cong (t - t_0)/t_0$ |
| Reduced viscosity | $\eta_{red} = \eta_{sp}/C$ |
| Inherent viscosity | $\eta_{inh} = (\ln \cdot \eta_r)/C$ |
| Intrinsic viscosity | $[\eta] = (\eta_{sp}/C)_{C=0} = [(\ln \cdot \eta_{red})/C]_{C=0}$ |