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# Optical and structural characterization of *Allium sativum* L. nanoparticles impregnated in beef tenderloin

## Caracterización óptica y estructural de nanopartículas de *Allium sativum* L. impregnadas en lomo de bovino

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## Abstract

To determine the optical and structural characteristics of garlic powder, garlic powder nanoparticles by size reduction were obtained and selection methods and their optical and structural characteristics were determined. The optical and structural response between commercial garlic powders and powders obtained by size reduction operations was compared. The particle size using transmission electron microscopy (TEM) images was determined; the optical characterization using Fourier transform infrared spectroscopy (FTIR) spectra was determined and, the structural characterization using X–ray diffraction (XRD) was carried out. Bovine tenderloin (*Longissimus dorsi*) samples were impregnated with garlic powder nanoparticles and the tissue was characterized by fluorescence technique. Powders obtained with size reduction and selection methods presented sizes values between 50 and 100 nm, while the normal garlic powder between 400 and 500 nm. FTIR spectra showed the presence of the main functional groups and X–ray diffractograms allowed to infer the structure of garlic as an amorphous material.

**Key words**: Garlic, nanoparticles, TEM, DRX, FTIR, fluorescence, bovine loin.

## Resumen

En el trabajo se determinaron las características ópticas y estructurales de nanopartículas de polvo de ajo (*Allium sativum* L.), obtenidas por reducción y selección de tamaño, para comparación con muestras de polvo de ajo comercial. El tamaño de partícula se determinó empleando microscopía electrónica de transmisión (TEM) y las caracterizaciones óptica y estructural se realizaron, respectivamente, por espectroscopia infrarroja transformada de Fourier (FTIR) y difracción de rayos X (DRX). Para determinar el efecto de las nanopartículas de polvo de ajo cuando fueron aplicadas sobre trozos de lomo (*Longissimus dorsi*) de bovino se utilizó la técnica de fluorescencia. Los tamaños de las partículas del polvo sometido a reducción y selección de tamaño presentaron valores entre 50 y 100 nm y las de polvo de ajo normal entre 400 y 500 nm. En los espectros de FTIR se observaron los principales grupos funcionales y los difractogramas de rayos X permitieron concluir que se trata de materiales amorfos. Por su tamaño más reducido, las nanopartículas migran más rápido al interior del músculo del bovino que las micropartículas, lo que permite una mejor absorción y aprovechamiento de sus componentes y se constituye en un resultado innovador en el campo de la ciencia de los alimentos.

**Palabras clave**: Ajo, especia, nanopartículas, TEM, DRX, FTIR, fluorescencia, lomo bovino.

## Introduction

Garlic (*Allium sativum* L.) belongs to the liliaceae family (García, 1998) has carbohydrates, sulfur compounds, proteins, free amino acids, phenolic compounds, fiber, minerals, saponins, moderate levels of selenium, vitamin A and C and B complex; additionally it is an important source of antioxidants (Lawrence, 2011). The medicinal properties of garlic are attributed to the sulfur groups, like the allicin and ajoene, biological active compounds that are formed upon maceration, cutting or cooking (Lanzotti, 2006). Allicin antimicrobial action is due to its activity as a strong inhibitor of some enzymes, among them the cysteine-proteases and alcohol dehydrogenases, which are responsible of the infections caused by bacteria, fungi and virus. Also, it has preventive and moderator effects in hypocholesterolemia, cardiovascular problems, high blood pressure; anti-inflammatory effects (Tsai *et al.*, 2012); it also has possible action mechanisms against cancer as mutagenesis and platelet aggregation inhibitor, reason why it is considered as anti-thrombosis material and with effects on immune-modulation (Chandrashekar and Venkatesh, 2009).

The nanotechnology is considered as the comprehension and control of particles from 1 to 100 nm in size, where some phenomena allow new applications (Chena and Yadab, 2011). In this measuring scale, physics, chemistry and biology fundamentally differ from the individual properties of atoms, molecules and materials when they are treated at higher scales, affecting the behavior of the physicochemical properties of the materials. The capacity to manipulate material at nano scale allows a better understanding of the biological, physical and chemical processes diversity, that, allows a better use of improved materials, novelty structures and electronic devices, among others (Sastry *et al.*; 2011).

Nanotechnology also has a wide range of action in sciences like medicine, electronics, food and agriculture. Its application has strengthen systems to control diseases in animals, water disinfection, safety in systems of food supply, energy conversion, plague localization technology, advances in fast detection of morphological changes in the physicochemical properties of food, increment in nutrient value, quality control, food security, development of novel products and packing design (Mahendra *et al*; 2009). The formation of nanoparticles, nanoemulsions and nanocapsules can improve the functional properties of the food products increasing their absorption in the body (Trujillo *et al*; 2013); its application allows the production of healthier and more productive foods, with better organoleptic properties, resistance and longer shelf life, benefiting the consumer, industry and opening new research fields.

The general objective of this research was to characterize optical and structurally nanoparticles from commercial garlic powder that were applied to beef (*Longissimus dorsi*) loin. The nanoparticles were obtained by the maceration and sieve technique and the characterization of the samples was done by the TEM, DRX and FTIR techniques.

## Materials and methods

### Nanoparticles isolation

The garlic nanoparticles obtained by the reduction and selection method in size, from 10 g of micro-particles of commercial 100% natural garlic powder were subjected to the following procedures: maceration for 6 hours using a vibrating micro-mill composed of a milling set (agate mortar and pestle) and, sieve through a Ro-tap model E test sieve shaker keeping the sample in constant movement for 15 min on a 20 µm sieve. Once the finer particles were filtered, for 2 hours the sample was macerated again.

### Particle size determination

The particle size determination at the Universidad Nacional de Colombia – Palmira using Transmission Electron Microscopy (TEM) was done. The garlic powder was dispersed on copper grill, placed on microscope specimen holders at 80 kilovolt and observed at 120,000X and 300,000X resolutions. The photomicrographs were observed on a monitor using a digital camera.

### Structural characterization

It consisted on hitting a beam of monochromatic X-rays on the sample, which interacts with radiation and gives precise information on the composition and crystallographic structure of the studied material. For that a diffractometer of X rays (DRX) Bruker D8-Advance coupled with an Cu anode tube that allows this type of radiation, a detector of 40 kV voltage and 40 mA current was used. The garlic powder was compacted on the specimen holder of the equipment and the measurements were performed on 3 – 60° angle, where each 3 sec 0.02° were measured being 2.5 h the average time to measure each sample.

### Optical characterization

The optical characterization of the garlic powder by Fourier Transformed Infrared Spectroscopy (FTIR) was done. In such technique the electromagnetic radiation in the infrared range is used to identify the composition of a material. The samples were compacted with a KBr dice in 1:10 proportion that was placed on an adapted cell in a spectrophotometer IR Prestige 21 Shimadzu. The sample spectra were obtained in wave number ranges from 4000 till 400/cm, that belong to the spectral range of the medium infrared (MIR).

### Characterization of beef loin samples impregnated with garlic powder

For this characterization beef loin pieces of 4 x 5 x 3 cm were taken. One part of them was impregnated with 0.1 g normal garlic powder (µPa) and the rest with nanoparticles of garlic powder (nPa) by covering or rubbing it in all the surfaces. The powder is partially dissolved and drained by the liquid effluent of the beef, as consequence of osmotic and diffusion mechanisms. To determine the fluorescence in the impregnated pieces with µPa and nPa a fluorescence inverted microscope EVOS was used; at the same time loin samples with or without impregnation of both garlic powders stored at 4 °C for 48 h were characterized. The observations were done on the fine cut surface of the loin samples placed on a specimen holder and read at 200 and 400 µm resolutions.

## Results and discussion

### Particle size

With the transmission electronic microscope (TEM) using a 120,000X resolution the Figure 1a and Figure 1b were obtained for normal garlic powder (commercial), where particles up to 200 nm width and 400 nm length were observed, with irregular shapes and high roughness. With 300,000X magnification were observed micrographs for the particles obtained by maceration, observing nanoparticles of 50 nm width and 100 nm lengths (Figure 1c and Figure 1d).

The above results showed that when the commercial garlic powder particles are subjected to maceration methods they reduce their initial size from 200 to 50 nm and present, on one side, a lower roughness by the effect of a mechanical force applied during the preparation process and, in the edges, nanochannels of unknown origin. On the

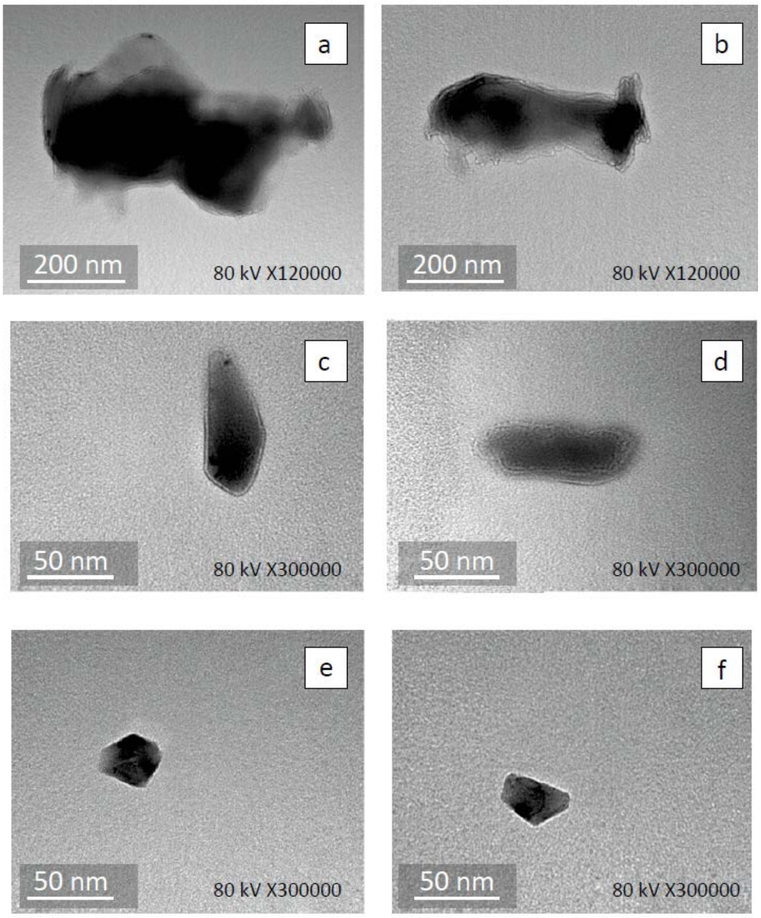


**Figure 1**. TEM Micrographs of the garlic powder surface: **(a)** and **b)** fine particles of commercial garlic at 120,000X magnification. **(c)** and **(d)** particles obtained by the maceration method at 300,000X magnification. **(e)** and **(f)** selected nanoparticles by the sieve and macerated method at 300,000X magnification.

observations with 300,000X magnification, the microphotographs of the nanoparticles allow the observation of particles of up to 20 nm in size with an irregular shape and disappearance of nanochannels, which can be attributed to the effects of an electrostatic interaction between the nanoparticles and the border of the metallic grid of the sieve (Figure 1e and Figure 1f).

## Structural characterization by X rays (DRX)

In the X rays diffractograms taken to commercial garlic powder and powder with size reduction (Figure 2) was observed a widely spread dominant peak, which is characteristic for amorphous materials. In the Table 1 are included the values of the highest peak and the full width at maximum half, where a slight movement of the peak towards higher angles is observed, together with a reduction on the width between the diffractograms of the powders subjected to reduction processes (maceration and sieved) and the commercial

**Table 1.** Values of the main peak and the full width at half maximum (FWHM) obtained from the analysis of powder garlic diffractograms.

| Particle type | Peak (degrees) | FWHM (degrees) |
|---|---|---|
| Normal garlic (μPa) | 18.46 | 11.84 |
| Macerated garlic (μPa) | 19.47 | 10.76 |
| Sieved and macerated garlic (nPa) | 19.14 | 11.17 |

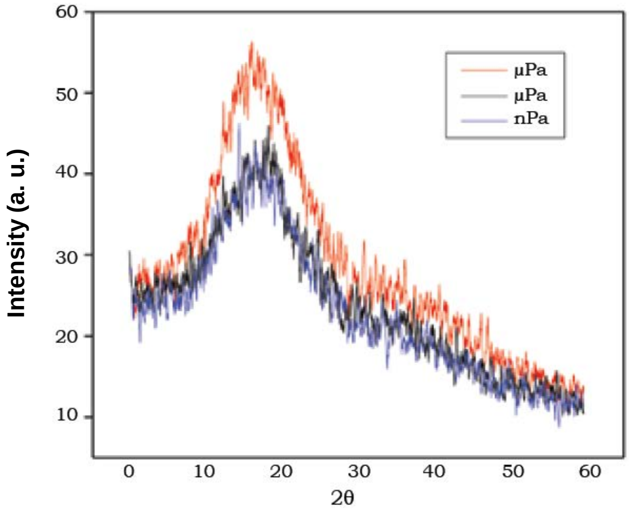


**Figure 2.** X rays diffractograms taken to the garlic powder samples. μPa = nanoparticles of sieved and macerated garlic. nPa = particles of commercial garlic powder.

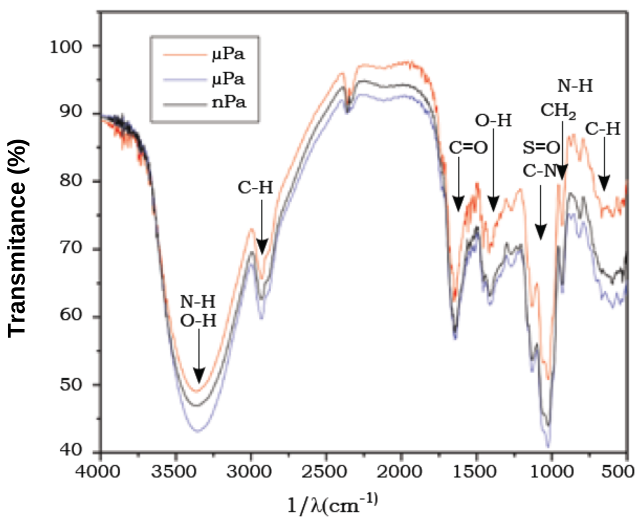


**Figure 3**. Characterizacion of functional groups by the FTIR technique. μPa = nanoparticles of sieved and macerated garlic. nPa = particles of commercial garlic powder.

garlic, which can be applied to the size reduction of the particles.

**Optical characterization by FTIR**

The optical characterization of the commercial garlic powder using Fourier transformed infrared spectroscopy (FTIR), following the protocols established by Skoog *et al*. (2001) to identify functional groups was performed. In the Figure 3 are presented the spectra for normal garlic powder and the one subjected to reduction processes and size selection. It can be observed that the structure of the spectra is independent of the size reduction processes and the functional groups are localized at the 3377/cm wavelength peak, which can be attributable to the stretching of the hydroxyl group O-H; while the 2931/cm peak corresponds to the stretching of the C-H bonds of the sp3 hybridization, the band at 1643/cm is assigned to carbonyls of different functional groups and, the peak in 1406/cm wavelength is assigned to the a O-H group of the carboxylate curve. The previous functional groups are possibly due to the high lipid concentration in garlic. On the other hand, the 1130 and 1107/cm bands can be attributed, respectively, to the sulfoxide absorption S=O and the stretching vibrations of the primary amines C-N, which are attributable to sulfur compounds like aliine and its derivates; the peak at 933/cm can be assigned to g-C-H deformation of $=CH_2$, while the one of 819/cm is to primary amine curves N-H that are the amine group of the aliine. Finally, the 605/cm peak can be associated with alkynes curve C-H of sp3 hybridization, as suggested by Rastogi and Arunachalam (2011).

**Fluorescence imaging**

The particles of normal (nPa) and nanoparticles (μPa) garlic powder emitted a high fluorescence (Figure 4a and Figure 4b, respectively). Due to the amount of garlic components, among them, minerals and sulfur compounds, this material showed high fluorescence allowing the determination of the impregnation and penetration degree when applied to the surface of beef loin (Figure 4c and Figure 4h). On the Figures is observed that the tissue impregnated with nPa shows a weak fluo-rescence, while in the muscle tissue impregnated with μPa there is no fluorescence. The former is due to fact that the nPa penetrate the muscle tissue after 48 h of storage in the fridge, allowing a better absorption and

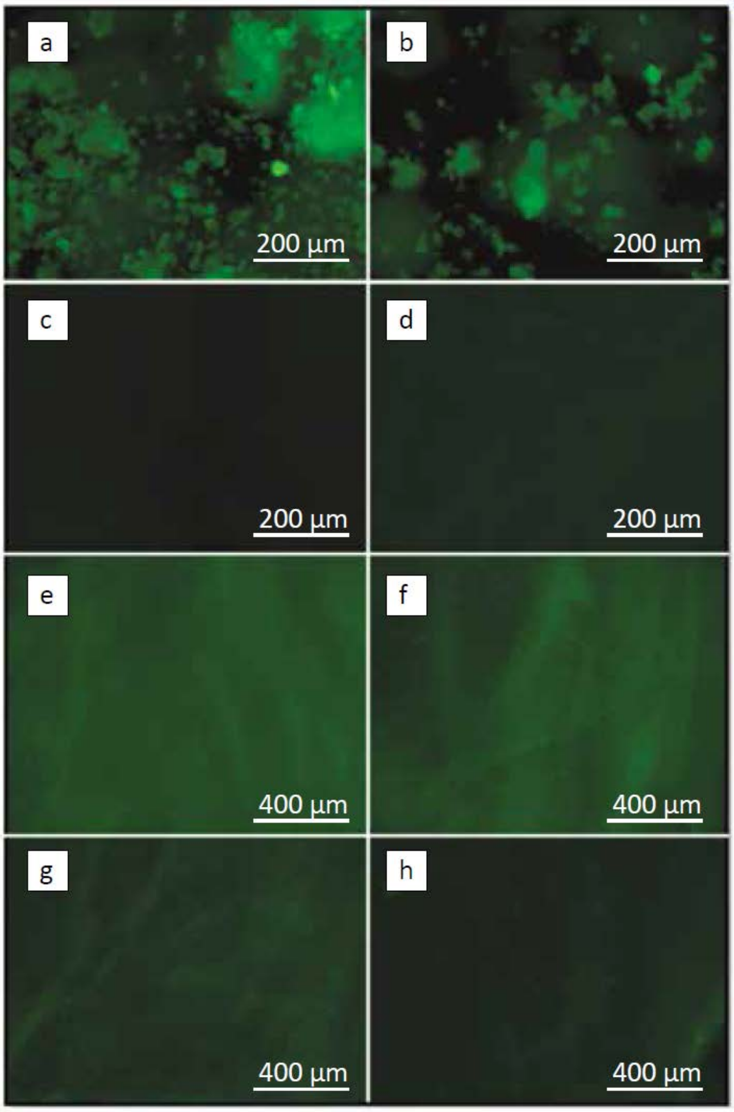


**Figure 4.** Fluorescence of garlic powder. (**a**) garlic nanoparticles; (**b**) garlic microparticles; (**c**) and (**d**) beef loin samples; (**e**) superficial cut of beef loin samples impregnated with nPa; (**f**) superficial cut of beef loin samples impregnated with µPa; (**g**) internal cut of beef loin samples impregnated with nPa; (**h**) internal cut of beef loin samples impregnated with µPa.

µPa = nanoparticles of sieved and macerated garlic powder.

nPa = particles of commercial garlic powder.

changes on the organoleptic properties of the muscle tissue of the bovine like flavor, aroma and tenderness.

Research on the preparation and systemic characterization of garlic nanoparticles are scarce, therefore, this is one of the first reports on the topic. Incorporation of nanoparticles in food started few years ago and currently there is little specialized information, which constitutes an open field for research. However, there are some related researches, among them the use of polyethylene-glycol nanoparticles covered with garlic essential oil with 240 nm in size as insecticide in the control of *Tribolium castaneum* adults (Feng–Lian *et al.*, 2009).

The results of this work showed that it is possible by reduction and size selection processes to get garlic powder nanoparticles. The size reduction could increase the particle specific area and its absorption capacity, which is innovative for food to enhance the garlic beneficial effects, which should be well used by its interesting nutritional and medicinal properties.

## Conclusions

- The reduction and size selection, as physical processes, did not alter the chemical composition of the nanoparticles obtained from commercial garlic powder.
- The Transmission Electron Microscope pictures allowed the identification of the nanoparticle formation obtained by reduction and size selection methods on garlic powder.
- The X ray diffractograms showed that the nanoparticles conserved the amorphous structure of the commercial garlic.
- The powder garlic nanoparticles, for its smaller size, migrate to the interior of the bovine muscle faster than the microparticles of the commercial garlic powder, allowing a better absorption and use of its components.

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