Application of nanotechnology in the production of nanoparticles of bioactive compounds: Case of rosemary (*Rosmarinus officinalis* L.) extracts

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ABSTRACT

Objective: To compile available information on techniques used in the production of nanoparticles involving functional compounds.
Design/Methodology/Approach: Published information and experiences from researchers working in the field of nanoparticles were compiled. Study protocols were created, and the advantages and disadvantages of nanoparticle fabrication were analyzed.
Results: The compiled information enabled the development of a flowchart for preparing nanoparticles from extracts containing bioactive compounds.
Limitations/Implications of the Study: Very little information is available. Specialized laboratories limit the use of these techniques.
Findings/Conclusions: A theoretical framework was developed to enable the use of nanoparticle fabrication techniques for research and to develop solutions that improve the shelf life of food intended for human consumption.

Keywords: bioactive compounds, nanoparticles, rosemary extracts, review.

INTRODUCTION

Nanotechnology has gained relevance in the food industry, particularly for enhancing antimicrobial and antioxidant properties and as an innovative alternative for food preservation. Chitosan-based nanoparticles have been used as vehicles for incorporating

various active substances due to their biocompatibility and non-toxicity. Their use in active packaging or in foods of animal origin directly impregnated with them has been employed to prevent lipid oxidation (Morales *et al*., 2024).

Chitosan is a derivative of chitin, one of the most abundant polysaccharides, second only to cellulose. Chitosan is composed of three reactive functional groups: primary and secondary hydroxyl groups at C-3 and C-6, and an amino group at the C-2 position. It consists of N-acetyl-D-glucosamine and glucosamine units and is formed from the partial deacetylation of chitin, resulting in a copolymer. Chitosan can be obtained through three methods: physical, chemical, and microbial. Once obtained, the resulting product is non-toxic, colorless, and tasteless. In nature, chitosan is found in the form of chitin in the shells of marine organisms, such as crustaceans, in eggshells, and in the mucilage of silkworms (Wang *et al*., 2024). Chitosan nanoparticles have a wide range of applications in various areas.

One line of research within our group has investigated the incorporation of rosemary extract into nanoparticle encapsulation and evaluated its functional activity. Rosemary extract (*Rosmarinus officinalis* L.) has been extensively studied because its properties make it a natural additive for food, as it can both improve and prevent lipid oxidation in meat. This is due to its content of bioactive compounds, such as flavonoids, terpenes, and the antioxidant rosmarinic acid (De Lima *et al*., 2024).

The concept of nanoparticles

The history of nanoparticles dates back to China and Egypt, where they were used for healing and aesthetic purposes. Subsequently, numerous studies have evaluated nanoparticles, leading to the emergence of disciplines dedicated to their study, including nanomedicine, nanotechnology, and nanoscience. These fields generally encompass the 1-1000 nm range, and their characterization has been made possible through the use of electron microscopy and X-ray absorption spectroscopy (Reynaud *et al*., 2020). The use and application of nanoparticles across areas such as medicine, cosmetics, and, more recently, food are advancing rapidly, and an increasing body of information is available to elucidate their mechanisms of action. The materials used in the production of nanoparticles must fulfill specific functions that provide them with particular properties and characteristics.

Application of nanoparticles

The study of nanoparticles has led to new applications in various fields, including food science and technology, which are now beginning to adopt their use. Nanotechnology allows for the modification of matter and the control of systems at the nanoscale (Ojeda *et al*., 2019). In the food industry, the use of appropriate packaging and coatings has become a topic of considerable interest due to their potential to extend the shelf life of many food products, enhance food quality, and reduce packaging waste. In this regard, there is an incentive to explore new bio-packaging materials based on renewable resources. Therefore, the application of nanocomposites (obtained from natural extracts) in packaging promises to open new possibilities for improving not only the properties but also the efficiency, contributing to the preservation of fresh food by extending its shelf life, as well as reducing packaging waste. Due to the nanometric size of the particles obtained by dispersion, these natural nanocomposites may represent an advance over conventional polymers (Marques *et al.*, 2012).

Extraction methods (bioactive compounds)

There are many alternatives when determining the method for extracting extracts of interest or essential oils. All techniques aim to preserve the components as much as possible; however, variables such as temperature, heat, energy, and time during extraction can lead to the loss of compounds of interest. The most commonly used techniques today are cold pressing and hydrodistillation (Bravo *et al.*, 2025). Other methods rely on mechanical forces for extended periods. For example, the solid-liquid method and the Soxhlet method are used to separate volatile compounds, but these have their disadvantages. They often pose health risks due to solvent use, and their components are heat-sensitive and may be lost during processing. On the other hand, ultrasound-assisted extraction demonstrates good performance in preserving phenolic compounds and, like microwaves, supercritical fluids, and solventless extraction, offers alternatives to traditional methods, yielding higher-quality extracts (Ramón *et al.*, 2021).

Physicochemical characterization of the extracts

The characterization of the extracts comprises a series of physicochemical tests whose primary purpose is to evaluate the compounds obtained during extraction. These tests include evaluation of antioxidant capacity using the DPPH and ABTS radical-scavenging methods and the iron-reducing antioxidant power (FRAP) assay. Additionally, the evaluation of phenols, flavonoids, and tannins enables the determination of these compounds and their quality (Manzanarez *et al.*, 2022). The nanoparticles are in the 1-1000 nm range; their size determines absorption, distribution, and accumulation. Functions associated with stability are also determined.

Principles of ionic gelation

The ionic gelation or ionotropic gelation method is used in the production of nanoparticles. It is based on electrostatic interactions between oppositely charged components, which form one or more ionic polymers. Chitosan and carboxymethylcellulose are the primary polymers used, along with stabilizing agents such as sodium tripolyphosphate (Hoang *et al.*, 2022). Other factors that stabilize the solution include pH and the physical forces of agitation. Furthermore, the rate and order of ingredient addition during encapsulation must be carefully controlled to ensure optimal nanoparticle performance (Vodyashkin *et al.*, 2022). Nanoparticles produced using the ionic gelation method have demonstrated stability and good encapsulation efficiency. The ratio for incorporating the components in the case of nanoparticles made from chitosan and tripolyphosphate is 3:1, and some authors use 2:1, with an optimal pH of 4.7-4.8, in adjusted acetic acid solutions, the results of size and morphology (Van Bavel *et al.*, 2023).

Particle size and polydispersity

Jeevanandam *et al.* (2018) identify factors that influence nanoparticle morphology and size, enabling classification of nanoparticles by origin. These can be type II, derived from synthetic nanomaterials, and distinguished by their size and morphology. In 2D and 3D, sizes greater than 100 nm with well-defined structures are observed. The dynamic light scattering technique enables the determination of particle size and the polydispersity index. Yuan *et al.* (2022) describe the challenges of preparing chitosan nanoparticles using sodium tripolyphosphate and explain how the preparation method, pH, stirring rate, and stirring time influence the process. All of these factors affect the degree of protonation of chitosan and its degree of interaction, thereby influencing its size and morphology, which makes it difficult to standardize its size, distribution, and morphology.

Z-potential

The most important properties for determining colloidal systems are those determined by the electrical charges produced by the particles that make up the system. This system forms when two phases with opposite charges come into contact. Once the system is formed, the electrokinetic degree of its components can be evaluated. The zeta potential determines this degree of interaction (Romero *et al.*, 2022). The zeta potential is the potential difference or electrical charge between the dispersion medium and the layer attached to the dispersed particle in the solution. This value is directly related to the stability of the colloidal medium (Mancini *et al.*, 2016).

Properties of rosemary extract

Rosemary is a plant that has been used throughout human history for a wide range of purposes, including in the production of cosmetics, anti-aging agents, and dyes, as well as in gastronomy due to its aromatic and flavor properties. This plant is rich in compounds such as antioxidants, enzymes, phenolic molecules, and glutathione, compounds that prevent the auto-oxidation of unsaturated triglycerides and oxidative stress in the cells of living organisms (Flores *et al.*, 2020).

Rosemary plant extract contains essential oils, which have been used for medicinal and other applications because they contain volatile molecules with biological, antioxidant, antimicrobial, antiseptic, anticancer, analgesic, antinephrotoxic, antitumor, antihepatotoxic, antifungal, antimutagenic, anti-inflammatory, and sedative properties. There is a wide variety of secondary metabolites in this plant, which are found mainly in the essential oil and extracts. These are grouped into three categories: flavonoids, hydroxycinnamic derivatives, and terpenoids (monoterpenes, sesquiterpenes, diterpenes, and triterpenes) (Flores *et al.*, 2020).

Scientific experience in the preparation of nanoparticles with rosemary extract

The preparation of chitosan-based nanoparticles loaded with rosemary extract is carried out according to the procedure described in Figure 1. Subsequently, these nanoparticles are analyzed for size, morphology, polydispersity index (PI), and zeta potential. The rosemary-extract-loaded nanoparticles exhibit a size of approximately 295 nm, with a PI of 0.459 and a zeta potential (ζ) of +36.6 mV. The encapsulation efficiency should be greater than 60%, indicating a considerable fraction of the extract encapsulated within the nanoparticles (Figure 2). Table 1 shows the most common properties observed in the preparation of rosemary-extract nanoparticles.

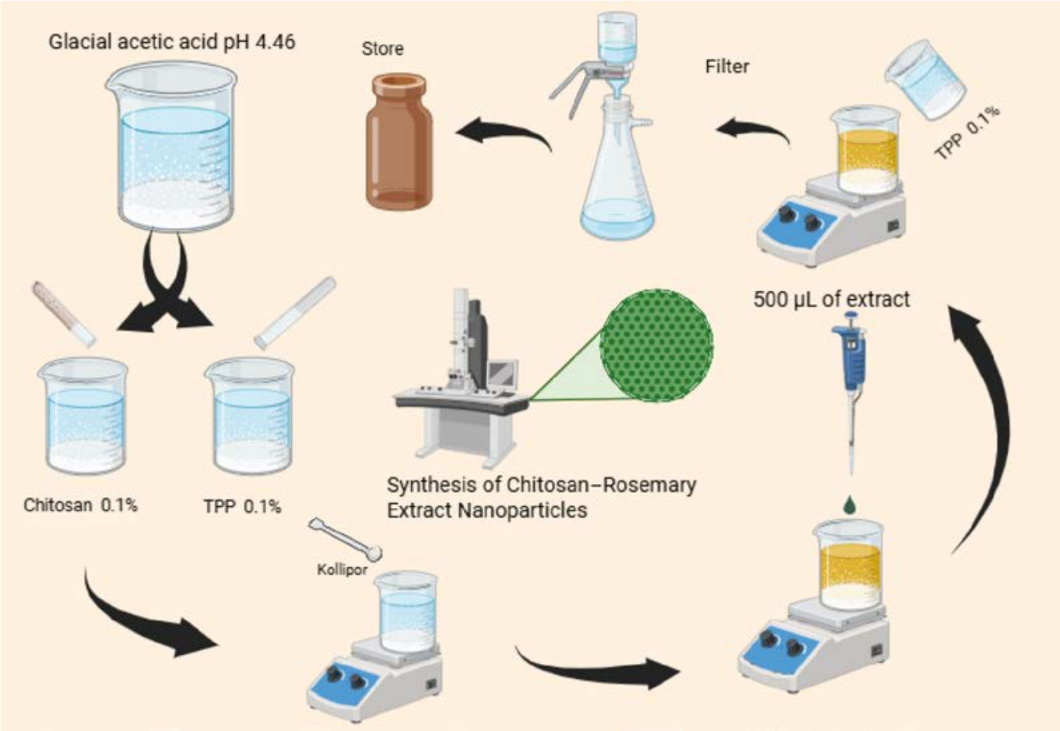

Figure 1. Procedure for obtaining nanoparticles.

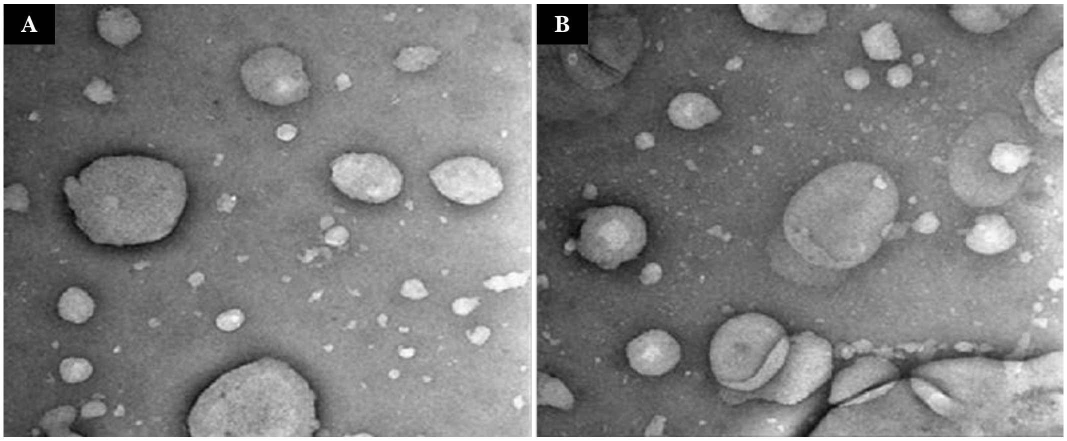

Figure 2. Transmission electron micrograph (TEM) of chitosan-based nanoparticles. (A) Nanoparticles loaded with rosemary extract (*Rosmarinus officinalis*) exhibit nano-oval morphology. With defined edges and a size distribution between 90 and 600 nm. (B) Empty chitosan nanoparticles with nano-spherical morphologies with less contrasting edges and a size distribution of 90 to 900 nm.

Table 1. Physicochemical characterization of the nanoparticles with rosemary extract.

Variable	Estimate	Unit	Brief interpretation
Size (peak intensity)	295	nm	Nano size is useful for coating
Polydispersity index	0.459	–	Moderate dispersion
Z Potential	36.6	mV	Positive charge; reasonable stability
Peak of the potential zeta	36.6	mV	Peak value in the distribution
z wall potential	30.5	mV	Auxiliary measurement of the equipment
Conductivity	0.664	mS/cm	Condition of the medium for ζ
Peak intensity of the area	95.7	%	Dominant mode (∼96% of the area)
Quality factor	5.8	–	Acceptable signal-to-noise ratio

Note. ζ=zeta potential. Units according to equipment output (conductivity in mS/cm).

The use of nanoparticles facilitates the application of extracts and essential oils by preventing the degradation and volatilization of compounds of interest and enabling controlled release. Studies such as that by Kasem *et al.* (2022) have demonstrated that the encapsulation of extracts improves the stability of phenolic compounds and their antioxidant activity. On the other hand, Hoang *et al.* (2022) mention that chitosan nanoparticles produced by the ionic gelation method typically have sizes greater than 100 nm, a value that is also linked to the molecular weight of chitosan; the lower the molecular weight, the smaller the nanoparticles. Other factors, such as time and agitation force, also influence the size. Specifically, the ionization potential (IP) explains the relationship between low values close to zero and their uniform dispersion in the solution, and vice versa. For the Z (ζ) potential, the values range from −100 mV to 100 mV and represent the colloidal stability of the nanoparticles through electrical charges. Jeevanandam *et al.* (2018) classify nanoparticles into type II nanoparticles derived from synthetic nanomaterials, and, according to their dimensions (size and morphology), they present sizes greater than 100 nm with well-defined structures.

Meat is a highly perishable food; therefore, it is more susceptible to contamination and spoilage by pathogenic microorganisms. Improper handling of meat, along with factors such as temperature, humidity, and storage time, influences the quality and safety of meat products. Another significant factor is its high-water activity (aw>0.95), which allows for the proliferation of microorganisms (Wornphan *et al.*, 2024). Currently, packaging is widely used to protect food from external factors that could alter its composition. It also provides safety by preventing damage during transport. Preservation extends shelf life, improves storage, and ensures proper handling (Bayona-Boneth *et al.*, 2023).

Meat is easily contaminated and is a perishable food because of numerous potential sources of contamination. Common examples include lipid oxidation, bacterial contamination, and enzymatic processes that reduce its shelf life, preventing its proper use. The industry relies on various tools to ensure adequate food preservation through temperature control during refrigeration. The use of polymers that serve as a physical barrier, preventing the entry of external contaminants, has also become more common (Kowalczyk *et al.*, 2024). Many materials are available on the market, the most commonly used for packaging being petroleum derivatives such as polypropylene (PP), high-density polyethylene (HDPE), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), expanded polystyrene (EPS), better known as Styrofoam, and flexible, soft polyethylene plastic film (PE). These materials are widely used for their desirable properties, including high strength, barrier properties, accessibility, and low cost (Momtaz *et al.*, 2024).

These act as barriers, enabling evaluation of changes in meat, such as moisture loss and color changes, thereby maintaining adequate control at each stage of the supply chain that affects the meat's shelf life (Brandelli *et al.*, 2024). The development of new packaging and preservation technologies is crucial for maintaining meat quality. In this case, the use of rosemary extract nanoparticles in vacuum-sealed packaging enhances meat quality and its physical and chemical properties, thereby helping to prevent lipid oxidation and providing antioxidant activity during storage.

CONCLUSION

Nanotechnology is the science that studies the design of functional and non-toxic materials for organisms. Its release systems enable the preservation and slow release of bioactive compounds, thereby maintaining their beneficial activity. This is the case with rosemary nanoparticles, which provide a means to preserve and release bioactive compounds, such as antioxidants, thereby extending the shelf life of meat.

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