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APPLICATION OF BIOGENIC SILVER NANOPARTICLES FOR BERRIES PRESERVATION

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The growing demand of consumers for healthy and quality nutrition is of a global nature. The modern development of technologies, including nanotechnologies, has made it possible to obtain bioproducts with unique properties that are being actively used in the food industry, but also require careful study of their properties and the impact on the human body and the environment. Silver nanoparticles (AgNps) are the most widely used nanoparticles (NPs) in the industry due to their antimicrobial properties. It is well known that silver can have a bactericidal as well as a bacteriostatic effect on many microorganisms. NPs obtained by biosynthesis are considered to be less toxic, safer, and cheaper to obtain. In this study, the objective was to obtain silver nanoparticles by the synthesis mediated with plant extracts (french marigold flowers (*Tagetes patula*), pot marigold flowers (*Calendula officinalis*) and lemon peel (*Citrus limon*)), aiming their practical application in fruits protection at storage, for a longer preservation.

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1. Introduction

The recent development of nanotechnology science has transformed many sectors of food industry such as processing, conditioning, packaging, storage, preservation [1], leading to increased quality and functionality. It is generally recognized that nanotechnology practical applications have the potential to revolutionize conventional food science and food industry.

Nanotechnology-assisted processing and packaging has already proved its beneficial effect in food systems [2]. As a consequence, the nanostructured materials are more and more used as additives for their microbicidal effect [3]. In particular, silver nanoparticles (AgNPs) and their derived nanocomposites as antimicrobial agent in food industry, has become interesting due to the inherent antimicrobial property of the silver. Nowadays, AgNPs are the most widely used nanoparticles (NPs) in the industry due to their antimicrobial properties [4, 5].

According to literary sources, it is known that silver can have a bactericidal as well as a bacteriostatic effect on many microorganisms (more than 500 species). Silver containing zeolites or other nanomaterials have official approval for use as food contact materials for the protection of packaged foods. AgNPs may be prepared using various physical, chemical and biological methods. AgNPs obtained by green synthesis are considered to be less toxic, safer, easily biodegradable, and cheaper to obtain [6, 7]. AgNPs have various uses as an antimicrobial agent: in textiles industry, water purification systems, medical devices, cosmetics and pharmacologic industry [7].

Plant extracts possess antioxidant activity due to the high content of polyphenols, especially anthocyanins [8]. Recently, plant extracts have been applied in the green synthesis of metallic nanoparticles. The biologically active compounds present in the extracts act on a double fold basis: as reducing agents for metal ions and stabilizers of the formed nanoparticles.

Various papers report the successful use of plant extracts for the synthesis of silver nanoparticles with significant antimicrobial activities: *Salvia spinosa* [9], bark of *Cinnamon zeylanicum* [10], olive leaf [7], *Ocimum tenuiflorum*, *Solanum tricobatum*, *Syzygium cumini*, *Centella asiatica* and *Citrus sinensis,* [11], *Vitis vinifera* [12], castor extract – *Ricinus communis*

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[13], onion [14]. In these studies, phytocompounds in the plant extract serve as reducing and/or capping agents in the reaction with silver nitrate ($AgNO_3$), a commonly used precursor in silver nanoparticle synthesis.

The application of film-forming compositions based on polymers on the surface of fruit and vegetable products allows increasing the shelf life [15, 16] slowing down the enzymatic processes of post-harvest ripening and aging of vegetables and fruits, as well as the development of many physiological diseases [17].

For the preservation of shelf life of soft fruits, various methods are used, such as: canning, freezing, drying, etc. As a rule, all these types of processing, which increase persistence, require considerable energy and lead to the loss of the nutritional value of fruits and vegetables.

The increased demand for minimally processed fruit las led to the need to identify innovative methods for providing fresh and ready-to-eat products. Technologies for conservation of qualitative parameters and innovative packaging of soft fruits are gaining momentum. The high perishability of fruit berries is given by the rapid decay of quality parameters and to increased microbiological deterioration. More and more, the consumers demand microbiologically safe, fresh, healthy, shelf-stable, convenient products produced using environmentally friendly technologies. In the above mentioned context, the present paper describes the results obtained by the treatment of berries fruits with biogenic AgNps, produced by green synthesis, with the use of vegetal extracts of french marigold flowers (*Tagetes patula*), pot marigold flowers (*Calendula officinalis*) and lemon peel (*Citrus limon*).

2. Materials and methods

2.1. Materials

All chemicals and solvents used in this study were of analytical grade and purchased from Sigma-Aldrich, unless stated otherwise. Distilled water was used throughout the study, where necessary.

2.2. Preparation of plant extract

Plant material was harvested from clean areas (*Tagetes patula* and *Calendula officinalis*) or purchased from the local vegetable market. Methods used for the preparation of plant extract samples and biosynthesis of AgNPs were adapted from literature and previous work of the authors on plant extracts with antimicrobial activity. Extracts were prepared by hot infusion, using a ratio of 10 : 1, vegetal material : water. The extracts were vacuum filtrated using Whatman no.1 filter paper.

2.3. Synthesis of AgNPs

Aqueous plant extracts have been used as a reducing agent in the extracellular synthesis of silver nanoparticles from an aqueous solution of silver nitrate ($AgNO_3$). The AgNps were prepared with constant amount of $AgNO_3$ solution, which was slowly mixed with the plant extracts on a magnetic stirrer. To resume, Constant amounts of plant extracts, and constant amount of silver nitrate with different concentrations was employed.

Briefly, an aliquot (10 ml) of aqueous plant extract was added to 100 ml of 1 mM aqueous $AgNO_3$. The reaction mixtures were exposed to direct sunlight for enhancing the formation of AgNPs. The change in color of the solutions was monitored. The NPs formation is indicated by a dark brown colour. Reaction solution obtained was kept for 24 hours for complete bioreduction and saturation. For further use, to prevent agglomeration of NPs, the containers were sealed and stored in dark, at room temperature.

Reduction of Ag^+ to Ag^0 was confirmed by changing the color of the solution from colorless to yellowish, brownish red and colloidal brown. Time and color changes were recorded along with regular sampling and scanning by UV visible spectrophotometry. This was done separately with each type of plant extract.

2.4. Sensory evaluation of berries

Fresh blueberries and blackberries were used in the research. The vegetal samples were divided into 3 groups and were sprayed with the nanoparticles solution or with deionised water solution (control tratment). The treated samples were kept at room temprerature, in dark, in closed plastic containers. Berry fruits were examined on a daily basis for the evaluation of the quality characteristics. The assesment was done on a 5-point scale (1 = inedible, 2 = poor, 3 = fair, 4 = very good, and 5 = excellent). The modification in quality parameters was observed and the experiment was performed until the visible symptoms of microbiological deterioration appeared.

3. Results and discussions

A primary indication of silver nanoparticle formation is represented by a reaction solution colour change to dark brown. Addition of $AgNO_3$ to plant extracts samples produced a colour change from an initial, yellow solution to dark brown solutions with all three extracts in 1 to 3h of reaction time. The UV-VIS spectra indicated the formation of AgNPs.

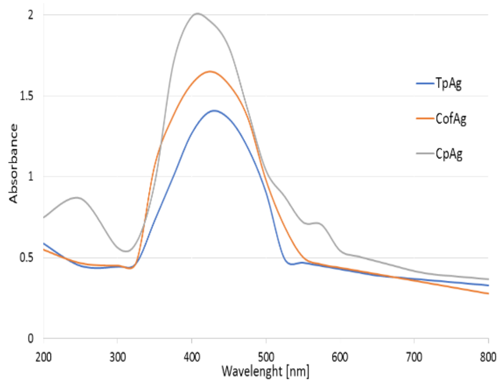

Fig. 1. UV-VIS spectra of biogenic AgNPs of 5 mM concentration.

Various processes associated with microbiological deterioration take place during storage of fresh horticultural crops (fruits and vegetables). They result from the harmful action of microbial organisms' growth which can be inhibited by action of antimicrobial agents, such as metallic Nps.

The evaluation of quality parameters of berry fruits was based on modification of odor, color texture and overall acceptability. Various concentrations of silver nanoparticles-plant extracts solutions were investigated for their effect on berries preservation. The berries species used were, *Vaccinium myrtillus* (blueberries) and *Rubus fruticosus* (blackberry).

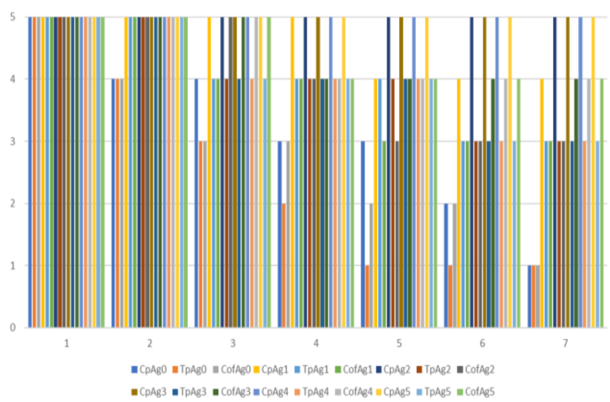

Fig. 2. Quality characteristics for blueberries over the entire storage period.

The preservation of quality parameter increased on a concentration basis of $AgNO_3$ used for NPs preparation. The influence of the mixtures on fruits preservation was evaluated by monitoring the sensorial and microbiological quality during the seven days storage period (Fig. 2.)

The most desirable results were observed for the citrus peel nanoparticles (CpAg), starting with 2 mM concentration. For the same concentration, the French marigold (TpAg) and pot marigold (CofAg) only resulted in a fair quality of the fruits coated with the AgNPs solution (Fig. 3).

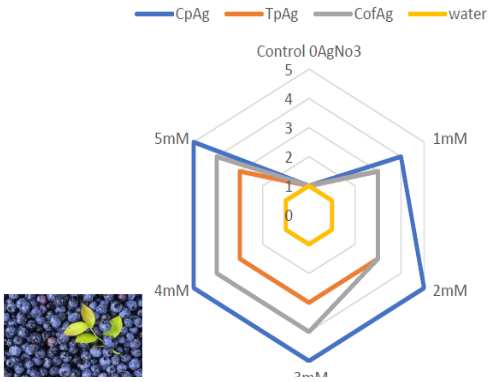

Fig. 3. Quality parameters for blueberry on a 5 point scale after 7 days.

The AgNPs obtained by green synthesis with citrus peel extract, were as well beneficial for the coating of blackberries and the preservation of quality parameters at room temperature (Fig. 4). Although not at the same level as CpAgNPs, the marigold extracts showed beneficial action on the microbiological and quality decay of the stored berries (Fig. 5).

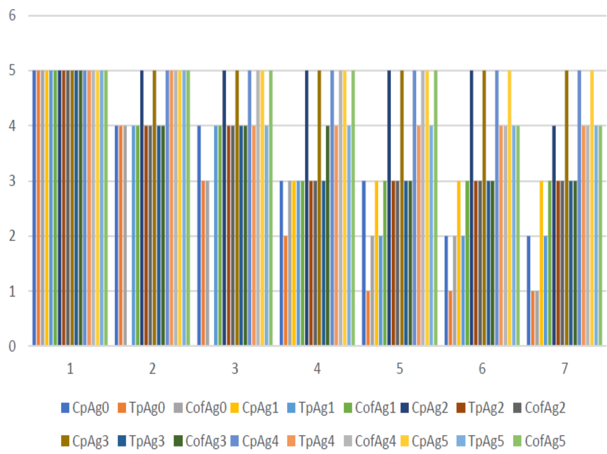

Fig. 4. Quality characteristics for blackberries over the entire storage period.

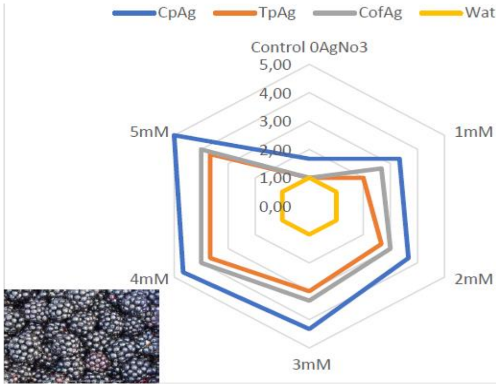

Fig. 5. Quality parameters for blackberry on a 5-point scale, after 7 days.

The synthesis of AgNPs mediated by plant extracts is considered to be simple, rapid, cheap, environmentally friendly, non-pathogenic, and possess a higher bio-reduction potential compared to microbial culture filtrates [18, 19]. Therefore, the use of plant extracts for producing nanoparticles is widespread because they are acting both as reducing and stabilizing agents for "green" biosynthesis of metal NPs.

The biogenic reduction is considered a "bottom up" approach where an extract of natural products with stabilizing and capping properties represents a reducing agent. Metallic nanoparticles can act as plant-growth stimulators, insecticides, and sterilizers [20]. Silver is involved in many practical applications because it possesses antimicrobial effect against microbial pathogens [11]. Other authors also reported on antifungal activity of AgNPs against phytopathogenic fungal strains such as *Colletotrichum gloesporioides*, *Bipolaris sorokiniana, and Magnaporthe grisea* as well as the plant pathogenic fungi *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *S. minor*, compared to commercial fungicides. For example, AgNPs mediated by *Acalypha indica* leaf extract presented antifungal effect against *Macrophomina phaseolina*, *Botrytis cinerea*, *Curvularia lunata*, *Rhizoctonia solani*, *Alternaria alternata*, and *S. sclerotiorum* [21]. Silver ions (Ag+) and AgNPs showed antifungal effect against to plant pathogenic fungi *Magnaporthe grisea* and *Bipolaris sorokiniana* [22].

4. Conclusions

The growing demand for minimally processed food lead to the constant challenge to develop innovative solutions for fruits preservation, based on antimicrobial bioproducts. Metal nanoparticles with antimicrobial activity can enhance the prolongation of fruits storage by inhibiting bacterial growth and providing a disinfecting action on the fruits surface. In this study, the silver nanoparticles produced by green synthesis with *Citrus limon* peel extract showed the best results on preservation of the berry fruit species tested.

However, it should be taken into account that the long-term environmental impact of NPs and effects on human health are major concerns among scientific groups, even for plant mediated synthesized nanoparticles. At this moment, there is no general consensus regarding beneficial vs negative effects of Nps and in-depth studies on the associated toxicological and environmental risks are within the target of future research.

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