ELABORATION OF BIODEGRADABLE WHEY ISOLATE FILM INCORPORATED WITH GREEN SYNTHESIZED SILVER NANOPARTICLES

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Abstract

Finding a feasible alternative to reduce the use of conventional polymers in the plastic sector has become a top priority, since industrially generated plastic waste, mostly traditional food packaging, has turned into a global environmental disaster. The aim of the study was the preparation and characterization of biodegradable films based on whey protein isolate, functionalized with green synthesized nanoparticles. Green silver nanoparticles (AgNPs) were synthesized using cinnamon extract as both capping and reducing agent. Biodegradable nanocomposite blend films based on silver nanoparticles and whey protein isolate (WPI) were made by casting denatured WPI film solutions incorporated with green synthesized silver nanoparticles. The film thickness and water vapor permeability (WVP) of both control and AgNPs were determined. The biodegradable films based on whey and (1%) green synthesized nanoparticles have potential application as active food packaging in the cheese industry.

Key words: whey, green synthesis, silver nanoparticles, biodegradable film, active packing

Finding a feasible alternative to reduce the use of conventional polymers in the plastic sector has become a top priority, since industrially generated plastic waste, mostly traditional food packaging, has turned into a global environmental disaster. Whey is the most common by-product of the cheese industry. Whey production in the globe is projected to be over 180 million tons per year, with the EU and the United States producing the majority (about 70%) (Babaei-Ghazvini *et al*, 2021).

In Romania, dairy producers pay additional prices for the destruction of whey, while in dairy factories abroad, it is appreciated and valued at a very good price. Romanian producers thus lose a huge opportunity to make a nice profit from the sale of whey, a by-product highly sought after by the dairy industry abroad (Spălățelu, 2012).

For human food or animal feed, about 50% of the world's whey is used. Some countries are stuck in the high cost of whey treatment, in expensive sewage treatment plants, even practicing the irrigation of pastures and agricultural land with whey or - simply - discharging the surplus into the oceans or streams. The discharge into the environment poses serious pollution problems, accentuated by the fact that one liter of whey has a biochemical oxygen consumption (BOC) of 50,000 mg/liter (Addai *et al*, 2020). Presently, the most rational and profitable way to capitalize on whey is to use it for human food and animal feed. However, in the past years, researchers have proposed the valorization of whey in the food package industry (Yadav et al, 2015; Di Pierro *et al*, 2017; Abdalrazeq *et al*, 2019; Pires *et al*, 2021). The utilization of the whey protein molecules as a polymer source for the manufacture of biodegradable plastic products may be a feasible way to recycle whey wastes.

The aim of the study was the preparation and characterization of biodegradable films based on whey protein isolate, functionalized with green synthesized nanoparticles.

MATERIAL AND METHOD

Reagents and materials

Commercial WP isolate (~90% dry basis protein) was purchased from ProNutrition (Dej, Romania), Glycerol and silver nitrate were purchased from Chemical Company (Iasi, Romania).

Green synthesis of silver nanoparticles

Silver nanoparticles were synthesized using ultrasound assisted cinnamon extract as both capping and reducing agent. Briefly, 10 grams of cinnamon bark powder (cinnamon zeylanicum) were mixed with distilled water and submitted to

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ultrasound treatment, at 60°C, for 30 minutes. Subsequently, the pH of the cinnamon extract was adjusted to 9 and an aliquot of 10 ml was mixed with 90 ml of silver nitrate (1mM) and submitted to heat treatment at 90°C, for 10 minutes. A change in color from light brown-yellow to reddish brown indicated the formation of silver nanoparticles. The synthesis of silver nanoparticles was confirmed by UV-VIS analysis. The size of the synthesized nanoparticles was determined according to an equation developed by Dalal et al (2019).

Preparation of AgNPs/WPI blend films

Film forming solution was prepared by dissolving WPI (5%, wt/vol) in distilled water and adding glycerol (5%, wt/vol). The solution was heated in a water bath at 90 ◦C for 30 min and rapidly cooled on ice to prevent further denaturation. Different amounts of green synthesized nanoparticles (1% and 5%, w/w) were added to the WPI film forming solutions. To control the dispersion of the AgNPs in the WPI polymeric matrix, the addition of AgNPs was carried out slowly and under intense stirring to prevent nanoparticle aggregation. The film forming solution incorporated with AgNPs was cast on 18.5-cm glass Petri dishes, previously coated with vege silicone solution (Elemental, Oradea, Romania). The WPI film solution was dried for 12 hours in an incubator at 37±0.5°C with a regulated relative humidity (RH = 40%). The dried films were then peeled off, visually inspected and kept at room temperature, between sheets of backing paper.

Determination of film thickness and water vapor permeability (WVP)

A micrometer was used to measure the thickness of AgNPs/WPI mix films (Parkside, Germany). The film thickness was determined by taking at least 10 measurements at random points on each film specimen and averaging the results. The water vapor permeability of the AgNPs/WPI films was determined using the capsule technique. The films were cut into 9 cm diameter discs and placed on top of a capsule containing 15 grams calcium chloride, ensuring that the moisture migration is only via the film. The cups were then placed in a desiccator at 25°C and 75% relative humidity. The weight increase of calcium chloride was monitored every 24 hours for 7 days to evaluate water vapor migration. The relative water vapor permeability (RWVP) of the elaborated films was calculated as follows: *RWVP=(m/t) x (1/A)*, where A is the permeation area of the film (m2), m is the mass of vapor permeated (g), while t is the time (days). The water vapor permeability (WVP) was calculated using the equation:

$$WVP=(RWVP \times e) /(p_s \times (RH_1-RH_2))$$

where e is the average film thickness (m), p_s is the saturated vapor pressure at the test temperature (Pa), RH_1 is the relative humidity inside the desiccator and RH_2 is the relative humidity inside the capsule.

At least three duplicates of each experiment were carried out. A one-way analysis of variance (ANOVA) was used to examine the data. All statistical analyses were conducted at a 95% confidence level.

RESULTS AND DISCUSSIONS

Silver nanoparticles were synthesized via biogenic or "green" route using ultrasound assisted extract as reducing and capping agent. The formation and growth of the synthesized nanoparticles was indicated by the color shift (*figure 1*). The synthesis of silver nanoparticles was confirmed by spectrophotometric analysis, at a resolution of 1 nm and wavelength range of 300-600 nm, a Surface Plasmon Resonance (SPR) peak being recorded at 407 nm (*figure 2*).

Figure 1 **Color shifting from yellow to reddish brown indicating the formation of green silver nanoparticles**

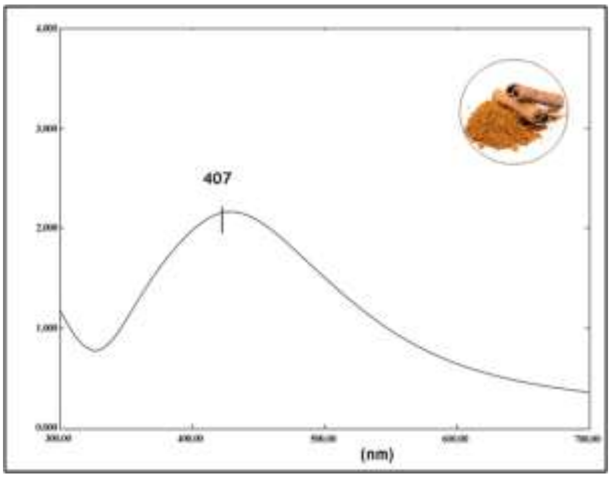

Figure 2 **UV-Vis Surface Plasmon Resonance of 'Green' Silver nanoparticles**

The size of the nanoparticles was determined using the equation developed by Dalal *et al* (2019).

$$AgNPs\ size = (SPR \times 0.84) -310$$

where SPR represents the recorded surface plasmon resonance. The estimated size of the green synthesized nanoparticles was 31.88 nm.

Further on, biodegradable nanocomposite blend films based on silver nanoparticles and whey protein isolate (WPI) were made by casting denatured WPI film solutions incorporated with green synthesized silver nanoparticles. The control film, containing only WPI and glycerol, were easy to detach from the plates, transparent, intact, with well-defined edges (*figure 3*). The film functionalized with 1%, respectively 5% green synthesized silver nanoparticles presented a light yellow color and and a faint hint of cinnamon. The film prepared with 1% green synthesized AgNPs were intact and presented slightly irregular margins (*figure 4*), while the one prepared with 5% green synthesized AgNPs presented visible cracks and pronounced irregular margins (*figure 5*).

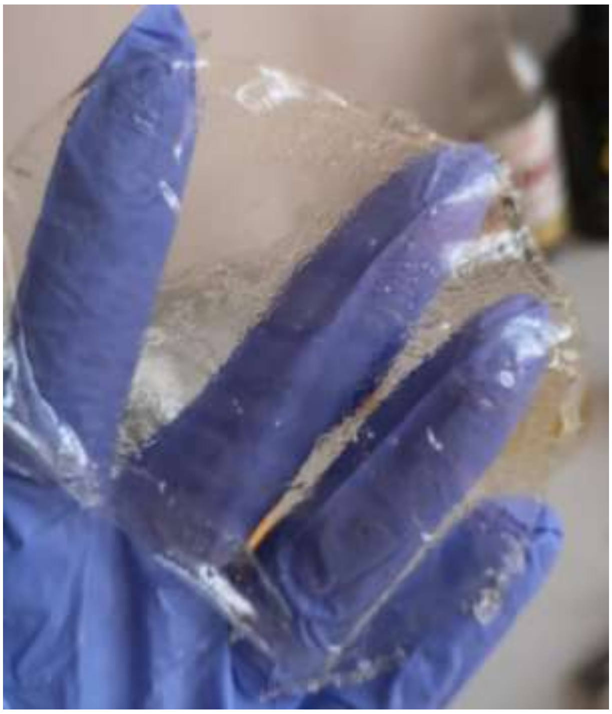

Figure 3 **Whey based control film**

The incorporation of AgNPs affected the elasticity and structure of the films, indicating the green synthesized nanoparticles may have an anti-plasticizing effect in WPI matrix, causing the functionalized WPI film to be more brittle and crack easier. Film thickness ranged from 0.0624 to 0.0864 um, the films incorporated with 5% green synthesized AgNPs preseting significantly lower thickness as compared to the control samples and the samples incorporated with 1% green AgNPs (*table 1*). The water vapor permeability (WVP) was significantly higher in the films incorporated with green AgNPs (*table 1*). It is likely that the green AgNPs didn't chemically attach to the protein matrix, allowing open gaps for water to pass through, resulting in a greater WVP in these films. The functionalization of whey based film through the addition of 5% AgNPs determined the matrix to destabilize, resulting in an increase in WVP. This problem was encountered in other studies conducted on WPI films incorporated with TiO2 nanoparticles. The authors came to the conclusion that the nanoparticle concentration in the WPI matrix influences the film's functions, such as WVP and mechanical characteristics (Zhou et al, 2009).

Figure 4 **Whey based film incorporated with 1% green synthesized AgNPs**

Figure 5 **Whey based film incorporated with 5% green synthesized AgNPs**

Table 1
Thickness and Water vapor permeability biodegradable whey isolate film incorporated with green synthesized silver nanopaticles

Type of biodegradable film	Thickness (mm)	WVP ($g.mm.m^{-2}.day^{-1}.kPa^{-1}$)
Control WPI	0.0864 ± 0.001[a]	23.74 ± 1.69[a]
WPI incorporated with 1% green synthesized AgNPs	0.0717 ± 0.001[b]	26 ± 0.05[b]
WPI incorporated with 5% green synthesized AgNPs	0.0624 ± 0.002[ab]	32.23 ± 0.90[c]

Mean (n = 3). ± Standard deviation a, b, c: Different letters in the same column indicate significant differences among AgNP concentrations in the WPC film (P < 0.05).

CONCLUSIONS

In order to be used in the economic zone, regardless of whether we refer to the food industry or to that of biodegradable plastic packaging, whey must be concentrated or fractionated, which implies further costs.

Due to the well know antimicrobial activity of silver nanoparticles, biodegradable films based on whey and (1%) green synthesized nanoparticles have potential application as active food packaging in the cheese industry.

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