Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.1590/fst.62720

Green biosynthesized *Satureja rechingeri* Jamzad-Ag/poly vinyl alcohol film: quality improvement of *Oncorhynchus mykiss* fillet during refrigerated storage

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Abstract

The nanosilver food packaging films were developed by polyvinyl alcohol activated with *Satureja rechingeri* extract (S-AgNPs/PVA) using the solvent casting to evaluate the freshness of *Oncorhynchus mykiss* fillets during 14 days at the refrigerated temperature. The 160 pieces of fish were enfolded in 4 types of films as main group-series. The main components of the extract were carvacrol (83.2%) p-cymene (3.11%), thymol (2.13%) and γ-terpinene (1.63%). The UV-Vis and TEM evaluations of S-AgNPs showed that the size of S-AgNPs was mostly smaller than 55 nm in diameter. Mesophilic and psychrophilic bacteria counts were remarkably less than 6.0 log CFU/g about 2.1, 1.3 log CFU/g and 2.0, 1.0 log CFU/g, respectively for fish samples wrapped in S-AgNP/Ult.PVA (Ultrasound method) and S-AgNP/Pho.PVA (Photochemical method) preserved at 4 °C on the 7th day. S-AgNP/Ult.PVA and S-AgNP/Pho.PVA showed strong efficiency in complete eliminating *S. aureus* and psychrophilic bacteria from coated trout samples than *E. coli* and mesophiles. It is suggested that AgNP/Ult.PVA and S-AgNP/Pho.PVA films can be used as nanocomposite film for fish preservation at refrigerated temperature to postpone the spoilage up to 7th day.

Keywords: green biosynthesis; *Satureja rechingeri*; nanocomposite; polyvinyl alcohol; fish; shelf life.

Practical Application: Films produced from Polyvinyl alcohol activated with *Satureja rechingeri* extract (both Photochemical and Ultrasound methods) and silver nano-particles can be applied as nanocomposite films in food-packaging industries to extend shelf life of fish fillets preserved at refrigerated temperature.

1 Introduction

The nanotechnology is extensively applying in the medicine and industries. The use of nanocombinations plays a crucial role in maintaining freshness and original nutritional attributes in food (Anvar et al., 2019). Metallic nanoparticles (MNPs) which have the antimicrobial potential can be applied in retaining the agricultural products such as fish, chicken, etc. (Lotfi et al., 2019). The biopolymers can be simply pooled with MNPs to produce the green combinations (Logeswari et al., 2015) to extend shelf-life of products, while they contact the products in the packaging (Santos et al., 2020; Singh & Danai, 2019). Among these products, the fish have a high degree of perishability; however, they specifically prone to rapid microbial spoilage, while they maintain high the freezing point (0.0 °C). At this circumstance, the fish carcass is not frozen, bacterial and enzymatic activities have increased, and affectedly shortened the shelf life (Paladini et al., 2019). To extend the shelf-life, fish should be kept under appropriate condition, in which the growth of bacterial spoilage is prohibited. natural compounds such as gelatin, and green nanocomposites are used to inhibit spoilage activities of bacteria (Wei et al., 2019). Nanocomposites are fabricated with silver nanoparticles/low-density polyethylene (AgNPs/LDPE) which can increase the shelf-life of fish at refrigerator temperature (Anvar et al., 2019), but for a limited storage time. Thus, prolonging the storage time for fresh fish preservation is a critical measure in the processing (Zulkarneev et al., 2019). Due to some evidence over the silver escaping from nanocomposite and worries concerning the recycling of LDPE as a host for silver ion and biocompatibility; however, the researchers are interested in either the green-synthesis of nanocomposites by reducing the concentration of silver and other ions in the final products (Dehghanizade et al., 2018), which produced from natural substrates such as tea polyphenols and chitosan composite film (Peng et al., 2020), edible film from mammee apple, *Mammea americana* a well-known tropical fruit (Nascimento et al., 2020), starch based film loaded with *Uncaria gambir* (Santoso et al., 2019), yam/maize starch edible film with lemon plasticizer (Song et al., 2019) or the environment-friendly polymer-based compounds as a host for ions (Nwabor et al., 2020). The herbal-derived nanoparticles (NPs) such as Savory (*S. rechingeri*) *extract* with remarkable antimicrobial activities can be applied in various professions such as health care, food industries, biomedicine and engineering sciences. Savory which belongs to the Lamiaceae containing more than 200 species of herbs (Navarro-Rocha et al., 2020) is one of the herbal medicine specifically rises in many countries in the Mediterranean area (Nwabor et al., 2020) and west of Asia where Iranian people have it daily as a side food or edible vegetable. It is well-known that it has great antioxidant properties compared to many vegetables, even higher than that of quercetin (Souri et al., 2004). Its extract can be used as a hybrid accompanying with the polymers (Mathew et al., 2018). Polyvinyl alcohol as the biodegradable

Received 13 Nov., 2020
Accepted 18 Nov., 2020
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polymer is a non-toxic, water-soluble, and transparent alteration of LDPE (Demir et al., 2019; Reddy et al., 2019) and can be activated with other natural compounds such as cellulose nanoparticles . Also, they have the appropriate obstacle characters to gases such as oxygen and carbon dioxide making its nanocomposite well-matched for fresh fish-coating in the packaging.

In this study, PVA based nanocomposite blend film incorporated with Aqueous extract of *S. rechingeri* and AgNPs was fabricated by solvent casting method. The nanocomposite blends were further characterized. Antibacterial and quality attributes were assessed while the *O. mykiss* fillets were encased in a different type of S-AgNPs/PVA films at refrigerated temperature for 2 weeks.

2 Materials and methods

2.1 Materials

Solid AgNO3 as lab grade, glycerol solution 86-89% (T), and PVA powder were prepared from Sigma-Aldrich (Germany).

2.2 The preparation of extract

The fresh Samples of *S. rechingeri* Jamzad (Savory) included leaves and flowers which their aerial parts were 100 g purchased from the grocery market, cut into small pieces, chopped, dried and put into a Clevenger glassware flask prefilled with 1.6 L of 2 times-ionized water (Pouya-Electric, Iran) and heated for 3 h at 80 °C. The ultimate extract with pale-yellow color was obtained and kept in the refrigerator to use.

2.3 Gas Chromatography-Mass Spectrometry (GC/MS)

The GC analysis was accomplished using Perkin-Elmer, Clarus 500 gas chromatograph, fixed with an HP 5MS 30 m × 0.25 mm × 0.25 µm film thickness capillary column and flame ionization detector. The column's temperature was initiated at the set from 60 °C and gradually increased to 280 °C at a rate of 3 °C a minute. The injector and detector temperatures were calibrated at 230 and 300 °C, respectively. Helium as the transporter was deployed at a unit of 1 mL/min. GC/MS analyses were accomplished with a Hewlett-Packard 5973-6890 GC/MS system functioning on electrospray ionization mode. Furthermore, the determination for each combination was confirmed by the evaluation of available marker samples (Alsaraf et al., 2020).

2.4 AgNPs synthesis

The 0.001 M silver nitrate ($AgNO_3$ — Merck, Germany) was combined with *S. Rechingeri extract* using two following procedures (Narchin et al., 2018):

1) Photochemical: $AgNO_3$ reagent as a precursor for silver and the original extract of *S. Rechingeri* were combined with a ratio of 1:4 to synthesize the combination of S-AgNPs. The sample was kept under the daylight for 5 min at pH=7. The rapid color alteration from light yellow to maroon implied making the AgNPs. Then, the samples were kept at room temperature for 24 hours;
2) Ultrasound: The previous proportioned combination for the initial phase of the photochemical process was introduced to ultrasound irradiation at 40 Hz in the dark condition for 30 min in triplicate at 40 °C and pH= 7. The changes in color from light yellow to maroon implied making the AgNPs.

2.5 Production of S-AgNPs/PVA films

The compounds deployed in this study were of the methodical grade. The S-AgNPs/PVA film was fabricated with macerated 5% PVA (w/v) powder (Merck, Germany) in hot 2-times distilled water and agitated at 80 °C for 1 h (Devi & Umadevi, 2014). The ultimate obtained solution was left at room temperature to be cooled, and the bubbles disappeared. The S-AgNPs reagent produced by two afore-mentioned methods individually was dripping into the flask prefilled with 0.75% glycerol as plasticizer and PVA solution at 65 °C for 25 min with slow stirring until a brown adhesive solution was appeared implying on the synthesis of S-AgNPs/PVA colloid (Figure 1). The finalized films were fabricated by the casting method (Nwabor et al., 2020) on a Teflon plate (Nippon Fusso, Japan).

2.6 Characteristic measurement

The prepared Ag/*S. rechingeri* were characterized by UV-visible spectroscopy, transmission electron microscopy (TEM), and X-ray diffraction (XRD). UV-visible spectroscopy is a crucial method applied to accept creating the metal nanoparticles in an aqueous solution. Therefore, producing the AgNPs was determined with scaling the UV-vis spectrum of reaction S-AgNPs. When the herbal extract became homogeneous in the solution of the 0.001 M silver nitrate, the reduction of AgNO3 into AgNPs in contact with plant extract was measured with UV-Vis spectrum (Shimadzu, Osaka, Japan) nad the absorption spectrum was recorded, ranging from 300-800 nm (Alaraidh et al., 2014).

The crystal structure of the produced Ag-NPs was examined with analysis of X-ray diffraction (XRD) patterns obtained from a diffract meter system (PANalytical, XPERT-PRO) The XRD patterns were recorded at a scanning speed of 4°/min (Jaiswal et al., 2020).

The scattering quality and size of S-AgNPs was examined using the Transmission electron microscopy (TEM) micrographs CM120 system from PHILIPS Co., Netherlands (Emamifar et al., 2010).

2.7 Study design

The whole live *O. mykiss* (15 kg) with an average body weight of 220 ± 43.20 g were purchased from the fish market and transferred to the microbiological lab with a distance less than 1h, eviscerated, washed with cold water and cut in some pieces. The 160 pieces of fresh *O. mykiss* fillets with an average weight of 50 ± 2.3 g were assigned to 4 groups (Table 1) based on a factorial design of three factors as 4×2×4 (Types of extract included control containing Aqueous, S-AgNP/Ult.PVA and S-AgNP/Pho.PVA; the storage temperature of coated fish fillets at 4 and 8 °C and finally sampling days of 1, 3, 7 and 14, respectively). The fish samples were enclosed in plasticized PVA stored in two

Figure 1. Ultimate produced Savory-Ag/Polyvinyl alcohol nanocomposites.

Table 1. The group assignment for experiment.

Coating/Nano Production	Temp. °C	Days	Group N0.
Control	4	1	G1
		3	
		7	
		14	
	8	1	G2
		3	
		7	
		14	
S-Aqueous	4	0	G3
		3	
		7	
		14	
	8	1	G4
		3	
		7	
		14	
S-AgNP/Ultrasound	4	1	G5
		3	
		7	
		14	
	8	1	G6
		3	
		7	
		14	
S-AgNP/Photo chemical	4	1	G7
		3	
		7	
		14	
	8	1	G8
		3	
		7	
		14	

refrigerators, adjusting to 4 and 8 °C up to 14 days (Figure 2). The group-series S-AgNPs Ult.PVA and S-AgNPs/pho.PVA were named for the fish coated with S-AgNPs/PVA nanocomposite film fabricated through ultrasound (Ult.) and photochemical (Pho.) methods, respectively. In addition, S-Aqueous and control group were named for the group-series in which the fish samples were covered with S/PVA (excluding AgNPs) and PVA films (excluding S/AgNPs), respectively (Table 1).

2.8 Bacterial analysis

The 25 g of *O. mykiss* fillets were added to 225 mL of buffered peptone water 0.1% (Merck, Germany) and homogenized in a stomacher (lab blender 400, Italy) at 260 rpm for 30s to obtain the colony-forming unit (CFU)/g for mesophilic bacteria count (MBC). Thereby, a ten-fold serial dilution was carried out in the peptone water, 1 mL of each dilution (initial suspension) pour plated with Plate Count Agar (PCA, Merck) in triplicate and finally incubated at 35 °C for 48h to count mesophilic bacteria.

Simultaneously, the psychrophilic bacteria count (PBC) was carried out. The 1 mL of final dilutions was added to the surface of triplicated plates pre-filled with Plate Count Agar (Sigma Aldrich, Germany) and incubated at 7 °C for 10 days (Ceylan et al., 2018).

The evaluation was continued with *E. coli* enumeration. The 3-tubes most probable number (MPN) method was applied to enumerate *E. coli* following Ramires et al. (2020) method with major modification. The serial dilutions of 10^{-1} -10^{-3} were made using the initial suspension. Three series of tubes (3 tubes) prefilled with 10 mL of Lauryl Sulfate Broth (Sigma-Aldrich, Germany) was used so that 1 mL of each dilution was added to the tube of each series and incubated at 37 °C for 24-48h. Due to the instruction of manufacturer, Turbidity of broth and gas formation were the clues for the presence of *E. coli* and/or other coliform organisms. Consecutive incubation phases were completed (in order) with inoculated EC broth and Trypton water (Oxoid, UK) at 44 °C for 24-48h, while the gas presence in Durham tubes was observed.

The biochemical test for *Salmonella* spp. growth was carried out with the incubation at 37 °C for 18 h using Bismuth Sulfite Agar (BSA) and Xylose Lysine D xycholate (XLD) agar (Sigma-Aldrich-Germany).

All plates with colony-forming units between 30 and 300 were recorded. The plates containing values greater than 300 were counted with colony meter applying the method of Khoshbouy Lahidjani et al. (2020) with major modification. To enumerate quagulase-positive *S. aureus*, the 1 mL of final bacterial suspension was inoculated on the surface of Baird Parker agar (BPA Sigma Aldrich, Germany) which incubated at 37 °C for 24-36 h (Sarab et al., 2019).

The CFU/g of bacteria were numerated following Equation 1.

$$CFU/g = \frac{\Sigma c}{V\left[n1 + (d \times n2)\right] \times d} \quad (1)$$

when: Σc = Sum of the CFUs counted on plates of two consecutive dilutions; V = sample volume inoculated to each plate (mL);

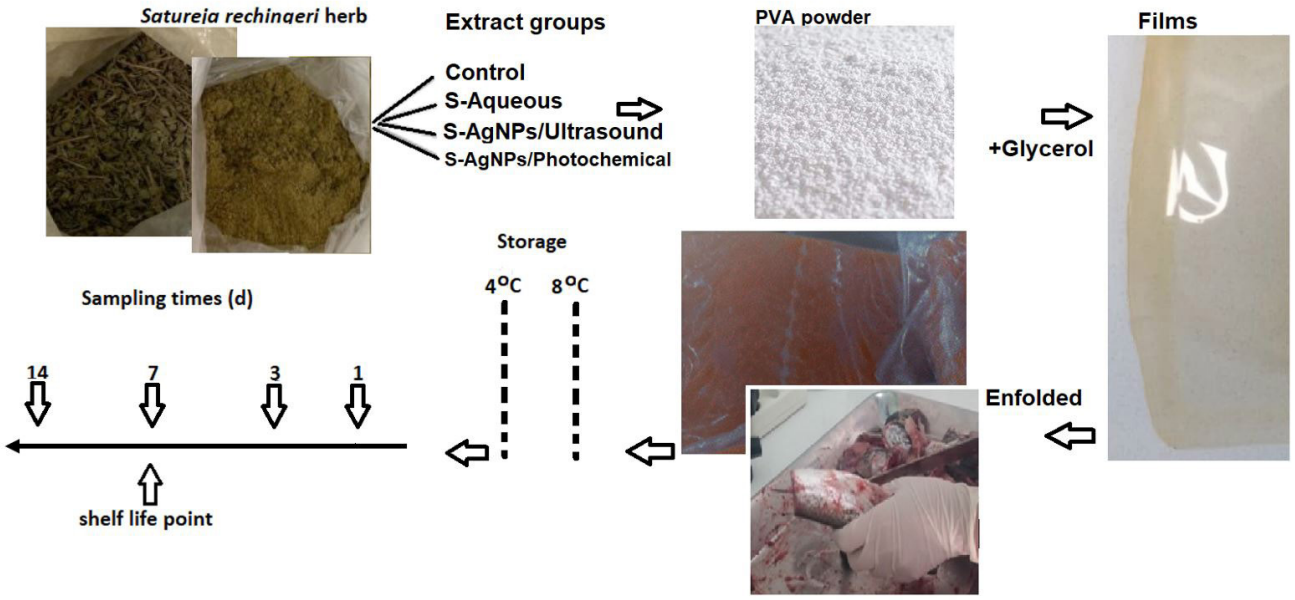

Figure 2. The Outline of experiment—the groups were controlled, *Satureja rechingeri-Aqueous* extract-PVA film, *Satureja rechingeri*-AgNPs/Ultrasound-PVA film, and *Satureja rechingeri*-AgNPs/photochemical-PVA film. *O. mykiss* fillets were folded in different films, stored at different degrees of temperature, and sampled in various days.

n1 = number of the selective plates for the first dilution; n2 = number of the selective plates for the second dilution; d = dilution coefficient concerning the first selective dilution.

2.9 Sensory assessment

The sensory assessment was done using one or more of five senses to evaluate the quality of fish enfolded in a different type of plasticized PVA at the time of sampling using the method of Diler & Genç (2018) with major modification. Trout samples were judged for appearance (skin brightness of fillets) color (fillet color changes), odor (sour, rotten), texture (fingermark disappeared immediately or within the time), and general acceptance (average score of main attributes). The 5-point hedonic technique was applied, and the fish samples were scored with values included 1 (excellent), 2 (good), 3 (acceptable), 4 (moderate) and 5 (weak), suggesting by panelists composed of 10 evaluators were trained following Nawaz et al. (2019).

2.10 Statistical analyses

Data of bacterial count were analyzed using a mixed model, ANOVA *repeated measure- Mixed model*. A factorial assignment was fixed to study the effect of 4 groups of the extract methods and two temperature of storage using 3 replicates. A Bonferroni test was carried out to compare the differences among the groups 2 by 2. No any non-parametric alternative to ANOVA-*repeated measurement* was accessible to analyze the qualitative data of organoleptic criteria, however, two independent variables including extract methods and temperature converted to one variable (Extract-Temperature) using compute approach in SPPS software and subsequently the Kruskal-Wallis *H* test was applied, which followed by Mann-Whitney U test. All data were analyzed using SPSS statistical software, version 26 (SPSS Inc., Chicago, IL).

Table 2. The most constituents of extraction, *Satureja rechingeri* Jamzad.

ingredients	*GC%*	
	Aqueous	*RI*
α-thujene	0.12	926
α-pinene	0.11	937
α-pinene	0.11	937
camphene	0.05	950
β-pinene	0.05	977
myrcene	0.20	988
α -phellandrene	0.15	1001
α -terpinene	0.41	1016
ρ-cymene	3.11	1021
γ -terpinene	1.63	1061
thymol	2.13	1288
carvacrol	83.2	1298
Eugenol	0.21	1354
caryophyllene oxide	0.27	1584
Sum	91.64	

RI = retention indices.

3 Results and discussion

The chemical composition of *S. rechingeri* Jamzad extract was evaluated by GC-MS. The results (Table 2) exhibited that the major components of the extract (90.07%) were carvacrol (83.20%) p-cymene (3.11%), thymol (2.13%) and γ-terpinene (1.63%) as Monoterpene combinations, which find in more than 100 herbs. The p-cymene is a well-known combination becomes the most important monoterpene finding in herbal medicines with antioxidant, antimicrobial, and anticancer properties as well as cytokine modulation (Marchese et al., 2017). The cell membrane of both Gram-negative and positive bacteria were meaningfully impaired after exposure to α- terpin l, γ-terpinene,

and eugenol (Oyedemi et al., 2009). Carvacrol is the major combination of *S. rechingeri* extract (Table 2) can chelate binding sites and reduce metal ions (Karmous et al., 2020) resulting a safe production of nanoparticles through green biosynthesis. Thus, the produced *S. rechingeri*/AgNPs nanocomposite can be act as safe antibacterial compare to AgNPs.

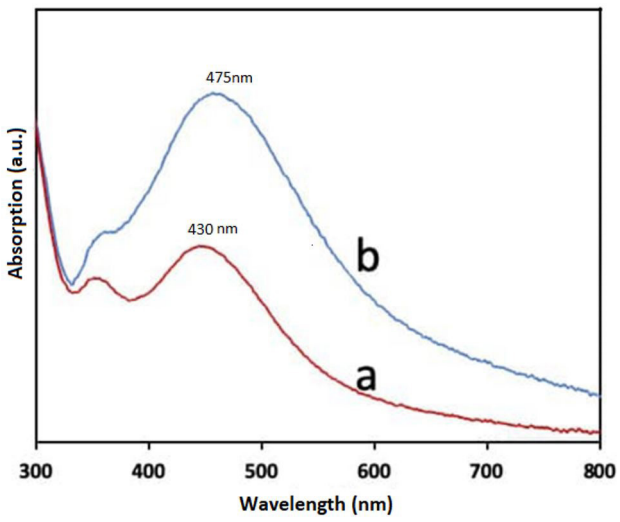

Figure 3. UV-Vis absorption spectra of *Satureja rechingeri*/AgNPs produced through ultrasound (a) and photochemical (b) methods.

Routinely, UV-Vis peak of AgNPs, which imply on the formation Ag-NPs, is in the range of 400-500nm depending on their size, shape and distribution in the aqueous solution (Gomaa, 2017). In the UV-Visible spectrum of produced S-AgNPs through the ultrasound and photochemical methods (Figure 3), the strong wide peak was detected at 430 and 475nm, respectively in agreement with the findings of Ashraf et al. (2016) exhibited the widening peak of AgNPs was performed at 455 nm. Broadening of the peaks (Figure 3) revealed that the AgNPs were polydispersed. Differences between the two peaks of wavelengths (Figure 3) confirmed also the TEM result (Figure 4), indicating smaller AgNPs produced through the ultrasound method than the photochemical one. TEM images display the size and dispersion of green synthesized AgNPs. The AgNPs diameter was different (Figure 4). These particles were well dispersed, with shapes of distorted spherical, spherical, hexagonal and irregular. More than half of the synthesized S-AgNPs/Pho had diameter size ranging from 35-55 but approximately 75% of S-AgNPs/Ult measured smaller than 30 nm. Due to a theory, lower wavelength indicates the greater released energy of a

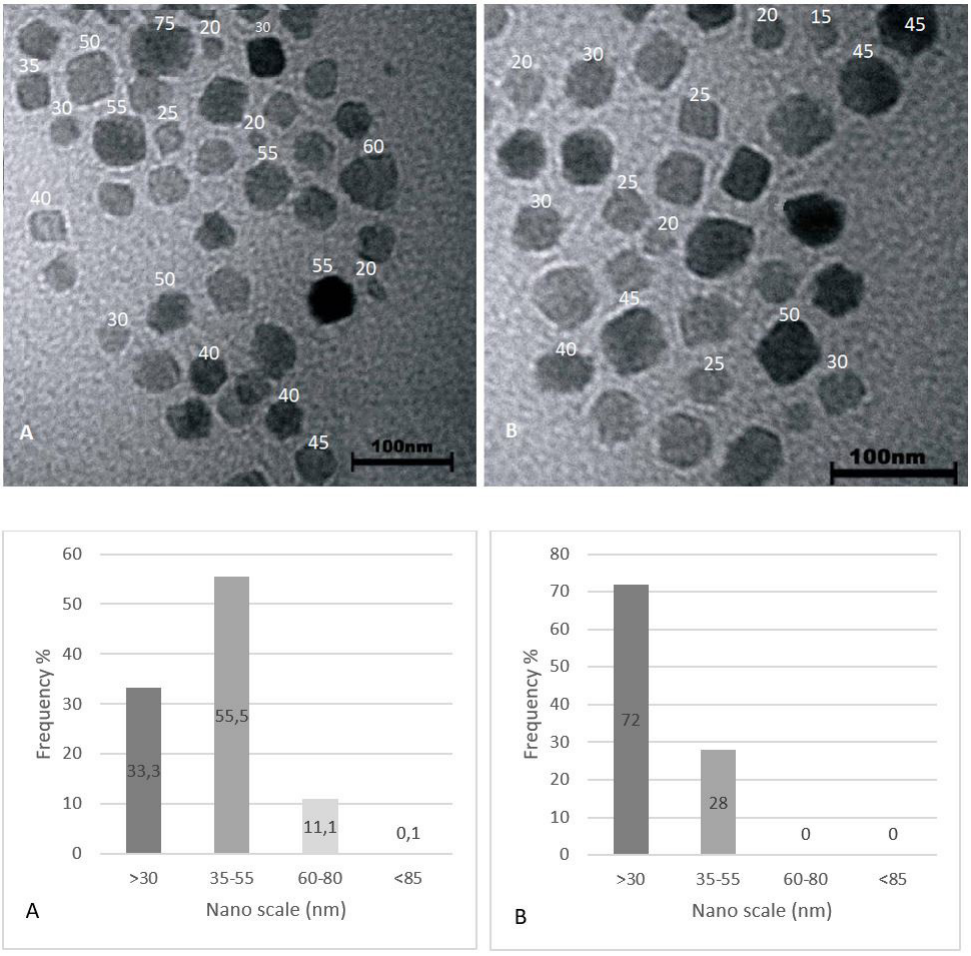

Figure 4. TEM shows the morphology of *Satureja rechingeri*/AgNPs produced through photochemical (A) and ultrasound method (B). S/AgNPs were chiefly distorted spherical in outline.

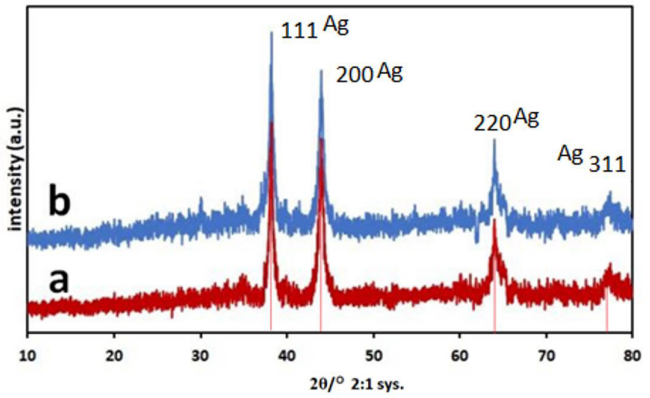

Figure 5. The XRD Pattern of green synthesized *Satureja rechingeri*/AgNPs peaks may be attributed to face-centered cubic 111, 200, 220, and 311 with 2θ angles. S-AgNPs produced through the photochemical (a) and ultrasound (b) methods.

wave, indicating smaller nanoparticles in the aqueous solution (Narchin et al., 2018).

Figure 5 shows X-ray diffraction (XRD) patterns of Ag-NPs implied on the crystalline arrangement. All S/AgNP samples, which their peaks were observed at 2θ values of 38.3182, 44.4975, 64.6119 and 77.5385 could be pertinent to 111, 200, 220, and 311 AgNPs-crystal structures. This pattern had a similar XRD outline compared to the standard powder diffraction card of Joint Committee on Powder Diffraction Standards (Theivasanthi & Alagar, 2012). The peak density of biosynthesized S-AgNPs revealed the high value of the crystallinity of AgNPs while the wide peaks indicating small size in crystallinity. In addition to the observed peaks (Figure 5), a few non-routine determined peaks could indicate the presence of other phytochemicals in mixture (Firoozi et al., 2016). The XRD pattern of S-AgNPs peaks (Figure 5) was exactly in agreement with another study (Gomaa, 2017), indicating an appropriate green synthesis of silver nanoparticles. This result (Figure 5) indicated that the application of the ultrasound method resulted in smaller nano-scale of AgNPs (Figure 5b), which in turn showed a stronger antimicrobial activity compared to larger ones (Sotiriou & Pratsinis, 2010). Such smaller nano-particles have greater contact surface area and release more Ag+ demonstrating great efficiency (Sotiriou & Pratsinis, 2010).

Antibacterial activity of the biosynthesized AgNPs is summarized in Table 3. The result showed, count of all bacteria (log CFU/g) obtained from all trout samples increased in a time-dependent manner. the maximum allowable limit (MAL) is 6 log CFU/g for MBC and PBC (Mol et al., 2007). According to the author's experiences (not published), spoilage bacteria are extremely activated after 5-7 days in rainbow trout preserved at refrigerated temperature. Generally, use of S-AgNP/Ult. PVA or S-AgNP/Pho.PVA resulted in MBC decrease less than MAL up to 7th day in fish preserved at 4 and 8 °C. Due to MBC normally grow at moderate temperatures between 20 and 45 °C, refrigerated temperature inhibited its growth well so that the MBC close to 6 log CFU/g is indicating the fish spoilage (Mol et al., 2007). The MBC load (Table 3) obtained from fish coated with S-Aqueous/PVA were significantly lower than those of control approximately by 1 log CFU/g for all sampling days, with the exception of the third day (4.55 ± 0.04 log CFU/g) showed a higher value ($p<0.05$) than that of control (3.70 ± 0.04 log CFU/g).

The lowest value of the MBC (1.42 ± 0.2 log CFU/g) was observed in G7 trout samples wrapped with S-AgNP/Pho.PVA films with no significant difference ($p>0.05$) compared to that of S-AgNP/Ult.PVA on the first day (1.65 ± 0.2 log CFU/g) at 4 °C. *Gadus morhua* fillets (Mizielińska et al., 2018) were wrapped with ZnO.NPs along with 2% polylysine postponed the growth of MBC reached 5 and 7 log CFU/g on 3rd and 6th day against this study, MBC were 2.68 ± 0.04 and 3.86 ± 0.05, respectively in fish samples coated with S-AgNP/Ult.PVA on 3rd and 7th d with no statistical difference ($p>0.05$) compared to those of samples were wrapped with S-AgNP/Pho.PVA (Table 3). Similarly, the MBC increasingly reached 5.5 log CFU/g in rainbow trout fillets coated with alkaline treated protein (AlPC) after 7 d at cold storage and surprisingly the slope was reduced after 7 d so that the MBC reached 6 log CFU/g on day 11 of cold storage (Özyurt et al., 2015).

On the 7th day (Table 3), MBC was remarkably decreased than MAL by about 2.1, and 2.0, log CFU/g, respectively for fish samples enclosed in S-AgNP/Ult.PVA and S-AgNP/Pho.PVA preserved at 4 °C while those of the control group (5.83 ± 0.05) showed no significant difference ($P>0.05$) compared to the MAL at the same conditions. The MBC reached 5.33 log CFU/g after 9d (Maghami et al., 2019) while the *Huso huso* fillets wrapped with chitosan-NPs activated with fennel essential oil (EO) relatively in agreement to this study showed the MBC was 3.86 ± 0.05 and 7.75 ± 0.02 log CFU/g in fish coated with S-AgNP/Ult.PVA with no significant difference ($p>0.05$) compared to those of S-AgNP/Pho.PVA on 7th and 14th day, respectively indicating that the MBC might be less than MAL (6 log CFU/g) and prolonged the shelf life up to 9th day, which was not analyzed in this study. Based on our previous study (Barani et al., 2018), LDPE/TiO2/Ag NPs could postpone the growth of mesophilic bacteria (6 log CFU/g) up to 14th day while its control reached this value on 6th day. The initial MBC (Barani et al., 2018) was less by 1 log CFU/g than that of this study (Table 3), which could be a justification for the difference between MBC values of both studies showing the similarity efficiencies of LDPE/TiO2/Ag NPs and the AgNP/PVA (both types) on reduction of MBC in fish fillets. The value of MBC of all treatments (G3-G8) were significantly ($p<0.05$) higher at 8 °C compared to those preserved at 4 °C.

Usually, the consumers are interested in preparing fresh fish preserved in refrigerated than frozen ones due to their nutritional values. The PBC becomes more important than MBC in the shelf-life quality evaluation (Quan et al., 2020; Seifzadeh & Rabbani Khorasgani, 2020). On the first day, the lowest value of PBC was observed (Table 3) in trout samples wrapped with S-AgNP/Ult.PVA (2.85 ± 0.02 log CFU/g) with no significant difference ($P>0.05$) compared to those of S-AgNP/Pho.PVA (3.04 ± 0.02 log CFU/g). The psychrophiles grew fast at 4 °C and no growth was observed at 8 °C after 3rd day of cold preservation, irrespective of the types of coating and sampling days. On the other hands, S-Aqueous/PVA showed the

Table 3. The effect of nano- extract fabrication type at various temperatures and days on the bacterial count (log CFU/g) of selected bacteria (n=3).

coating/Nano.P	T °C	Days	log CFU/g (Mean ± Standard Error)			*E. coli*
			Mesophile	Psychrophile	*Staphilococcus*	
Control		0	3.02 ± 0.2^{aA}	3.60 ± 0.02^{aA}	0.0 ± 0.00^{aA}	0.36 ± 0.03^{aA}
	4	1	3.17 ± 0.2^{aA}	3.86 ± 0.02^{aA}	0.0 ± 0.00^{aA}	0.39 ± 0.03^{aA}
		3	3.70 ± 0.04^{bA}	4.82 ± 0.04^{bA}	0.0 ± 0.00^{aA}	0.35 ± 0.05^{aA}
		7	5.83 ± 0.05^{cA}	5.83 ± 0.05^{cA}	0.77 ± 0.27^{bA}	1.55 ± 0.03^{bA}
		14	8.93 ± 0.02^{dA}	8.93 ± 0.02^{dA}	3.80 ± 0.08^{cA}	3.02 ± 0.03^{cA}
	8	1	3.16 ± 0.2^{aA}	3.86 ± 0.02^{aA}	0.0 ± 0.00^{aA}	0.39 ± 0.03^{aA}
		3	4.52 ± 0.04^{bB}	0.0 ± 0.04^{bB}	0.0 ± 0.00^{aA}	0.41 ± 0.05^{aA}
		7	6.41 ± 0.05^{cB}	0.0 ± 0.05^{bB}	2.16 ± 0.27^{bB}	1.62 ± 0.03^{bA}
		14	10.93 ± 0.02^{dB}	0.0 ± 0.02^{bB}	4.25 ± 0.08^{cA}	3.19 ± 0.03^{cA}
S-Aqueous	4	1	2.22 ± 0.2^{aB}	3.79 ± 0.02^{aA}	0.0 ± 0.00^{aA}	0.35 ± 0.03^{aA}
		3	4.55 ± 0.04^{bB}	4.75 ± 0.04^{bA}	0.0 ± 0.00^{aA}	0.35 ± 0.05^{aA}
		7	4.72 ± 0.05^{bC}	6.18 ± 0.05^{cA}	0.00 ± 0.27^{aC}	1.36 ± 0.03^{bA}
		14	8.57 ± 0.02^{cA}	10.83 ± 0.02^{dC}	3.00 ± 0.08^{bB}	3.05 ± 0.03^{cA}
	8	1	2.22 ± 0.2^{aB}	3.79 ± 0.02^{aA}	0.0 ± 0.00^{aA}	0.35 ± 0.03^{aA}
		3	4.51 ± 0.04^{bB}	0.0 ± 0.04^{bB}	0.0 ± 0.00^{aA}	0.35 ± 0.05^{aA}
		7	5.52 ± 0.05^{cA}	0.0 ± 0.05^{bA}	0.00 ± 0.27^{aC}	1.43 ± 0.03^{bA}
		14	10.82 ± 0.02^{dB}	0.0 ± 0.02^{bB}	3.70 ± 0.08^{bA}	3.15 ± 0.03^{cA}
S-AgNP/Ultrasound	4	1	1.65 ± 0.2^{aC}	2.85 ± 0.02^{aB}	0.0 ± 0.00^{aA}	0.32 ± 0.03^{aA}
		3	2.68 ± 0.04^{bC}	3.84 ± 0.04^{bC}	0.0 ± 0.00^{aA}	0.35 ± 0.05^{aA}
		7	3.86 ± 0.05^{cD}	4.70 ± 0.05^{cC}	0.00 ± 0.27^{aC}	0.92 ± 0.03^{bB}
		14	7.75 ± 0.02^{dC}	8.44 ± 0.02^{dA}	0.00 ± 0.08^{aC}	2.33 ± 0.03^{cB}
	8	1	1.65 ± 0.2^{aC}	2.86 ± 0.02^{aB}	0.0 ± 0.00^{aA}	0.32 ± 0.03^{aA}
		3	3.52 ± 0.04^{bA}	0.0 ± 0.04^{bB}	0.0 ± 0.00^{aA}	0.35 ± 0.05^{aA}
		7	4.63 ± 0.05^{cC}	0.0 ± 0.05^{bB}	0.0 ± 0.27^{aC}	1.06 ± 0.03^{bB}
		14	8.19 ± 0.02^{dA}	0.0 ± 0.02^{bB}	0.0 ± 0.08^{aC}	2.67 ± 0.03^{cA}
S-AgNP/Photo-chemical	4	1	1.42 ± 0.2^{aC}	3.04 ± 0.02^{aB}	0.0 ± 0.00^{aA}	0.30 ± 0.03^{aA}
		3	2.94 ± 0.04^{bC}	3.96 ± 0.04^{aC}	0.0 ± 0.00^{aA}	0.35 ± 0.05^{aA}
		7	3.98 ± 0.05^{cD}	4.95 ± 0.05^{bC}	0.0 ± 0.27^{aC}	1.14 ± 0.03^{bB}
		14	7.92 ± 0.02^{dC}	8.50 ± 0.02^{cA}	0.0 ± 0.08^{aC}	2.54 ± 0.03^{cB}
	8	1	1.42 ± 0.2^{aC}	3.04 ± 0.02^{aB}	0.0 ± 0.00^{aA}	0.30 ± 0.03^{aA}
		3	3.72 ± 0.04^{bA}	0.0 ± 0.04^{bB}	0.0 ± 0.00^{aA}	0.35 ± 0.05^{aA}
		7	4.80 ± 0.05^{cC}	0.0 ± 0.05^{cB}	0.0 ± 0.27^{aC}	1.30 ± 0.03^{bB}
		14	8.65 ± 0.02^{dA}	0.0 ± 0.02^{dB}	0.0 ± 0.08^{aC}	2.50 ± 0.03^{cB}

Extract/Nano.P: The production methods of *S. rechingeri* -Ag/NPs; T: Temperature. Different small superscripts in each extract group for each temperature indicate a significant difference ($p< 0.05$). To compare all groups, capital superscripts in each column, and the same days show a significant difference ($p< 0.05$).

unpredictable values in sampling days and had no any acceptable effect on reduction of PBC. It was significantly increased up to 14d (10.83 ± 0.02 lof CFU/g). On the third day of preservation, the least PBC was also observed in fish samples wrapped with S-AgNP/Ult.PVA films (3.84 ± 0.04 log CFU/g), and less than that of Aqueous/PVA composites by about 1 log CFU/g ($p<0.05$) at 4 °C and had no significant difference ($p>0.05$) compared to that of S-AgNP/Pho.PVA films at the same condition. After 7 d, The PBC of G5 samples (4.70 ± 0.05 log CFU/g) were less than the values of both control (G1), S-Aqueous/PVA (G3) by about 1.1 and 1,5 log CFU/g ($p<0.05$), respectively and group of S-AgNP/Pho.PVA (by 0.25 log CFU/g) with an insignificant difference ($p>0.05$). The PBC of trout samples incorporated with thymol nanoemulsion (Meral et al., 2019) at a nano-scale (smaller than 70nm) was reached 5.70 log CFU/g on 7th day at refrigerated temperature (from 3.95 log CFU/g on 1st day) greater than that of the trout samples (Table 3) enclosed in S-AgNP/Ult. PVA (4.70 log CFU/g) or S-AgNP/Ult.Pho (4.95 log CFU/g) with NPs almost scaled less than 30-35nm (Figure 4) indicating the smaller the NPs the greeter the antibacterial efficiency (Sotiriou & Pratsinis, 2010). Applying ZnO nanocomposite along with *Mentha spicata* could retard psychrophilic bacteria growth up to 7th day at the MAL level (Shahbazi & Shavisi, 2018a) higher than the findings of this study (Table 3) exhibited PBC reached 3.84 and 3.96 log CFU/g, respectively in rainbow trout fillets covered with S-AgNP/Ult.PVA and S-AgNP/Pho.PVA at 4°C. The psychrophilic microorganisms are responsible for off-odor occurred in the most spoilage of foods (Meral et al., 2019). Farsi gum-based coatings incorporated with clove and Shirazi thyme essential oil emulsions were used to prolong the shelf-life of rainbow trout fillets at refrigerated temperature. Despite the great bactericidal properties of the mentioned essential oils (Majdinasab et al., 2020), the resultant emulsion could not significantly decrease the amount of psychrophilic bacteria

even increase it so that the PBC incredibly went up from 1st (4-5 log CFU/g) to 4th d (more than 6 log CFU/g) more than the MAL (Shahbazi & Shavisi, 2018a), which could be due to the method of extraction or sonication (Dehghani et al., 2018). The sensory criteria showed the silver pomfret stored in ice could be edible up to 12th day, while the PBC was 6.0 log CFU/g on 3rd day and remarkably increased afterward (Fazlara et al., 2014).

The antimicrobial activities of AgNPs on Gram-negative bacteria was shown to be greater than Gram-positive bacteria which could be due to the less thickness wall of Gram-negative ones (Seong & Lee, 2017). Monoterpenes, the major ingredients (more than 90%) in *S. rechingeri*, inhibit the growth *of S. aureus* more than *E. coli* (Trombetta et al., 2005) in agreement with other studies (Alboofetileh et al., 2014; Alizadeh, 2015; Paredes et al., 2014; Pirtarighat et al., 2019) confirmed that *S. aureus* was more sensitive than *E. coli*. Similar to the findings of this study (Table 3), a new finding indicated that *S. aureus* was more sensitive to chitosan/PVA nanocomposite than *E. coli* (Hajji et al., 2016). Dislike, The S-AgNP/Pho extract showed greater effect on the both bacteria (same effectiveness) compared to S-AgNP/Ult one (Narchin et al., 2018). The afore-mentioned differences of the efficiency on the both bacteria may be due to a few factors such as size of AgNPs, types of nano-metals (Meral et al., 2019), bacterial resistance, growth phase of herbs, major composition of extract, and the experiment methodology (Alizadeh, 2015; Paredes et al., 2014). However, In the rainbow trout fillets were packed in chitosan-ZnO films containing different concentrations of pomegranate peel extract (1.5%), the value for *S. aureus* was 2.0 log CFU/g at 6d, which were eliminated from the fillets on 7d at 4 °C (Shahbazi & Shavisi, 2018b) a one week delay in comparison to this study (Table 3), which could be due to the different use of extracts, because the volume of NPs required for *S. aureus*-growth inhibition impacted by ZnO and Ag nanocomposites was usually the same (Salem et al., 2015). The growth of *S. aureus* neither observed in the trout fillets coated with S-AgNP/PVA-Ult nor in those of S-AgNP/PVA-Pho films during the study (14d). Against the control group on which the occurrence of *S. aureus* was began on 7th day (0.77 ± 0.27 log CFU/g at 4°C), *S. aureus* was observed from the 14th day in the fillets of S-Aqueous groups, so that the values were 3.00 ± 0.08 and 3.70 ± 0.08 log CFU/g, respectively at 4 and 8 °C ($p<0.05$). On 12th day, *S. aureus* count was reached 3.38 ± 0.05 log CFU/g in the fish covered with PVA-gelatin films incorporated with *Amaranthus* leaf extract (Kanatt, 2020) greater than those of the fish samples of this study even in S-Aqueous/PVA group (Table 3).

Regardless of whether the S-NPs were incorporated with PVA or what temperature degree was applied, *E. coli* count was increased with a time-dependent manner up to 7th day so that the least value was measured in G7 on the first day (0.30 ± 0.03 log CFU/g) with insignificant difference ($p>0.05$) compared to those of other groups. Dislike, poly sulfobetaine methacrylate along with bacterial nanocellulose could decrease *E. coli* count by 1.1 log CFU/mL after 24h (reached 8.5 log CFU/mL) but showed no effective reduction (Vilela et al., 2019). Dislike, alginate/carboxyl methylcellulose nanocomposite film along with clove- EO (1.5%), a combination without Ag^+ ions, could significantly decrease *E. coli* counts in the silver carp fillet from 3 log CFU/g at first day to approximate 1 log CFU/g on 12d (Jalali et al., 2016). On 7 and 14d, trout samples of G5 coated with S-AgNP/Ult-PVA films had the lowest *E. coli* load, while it reached 0.92 ± 0.03 and 2.33 ± 0.03 log CFU/g, respectively less than those of control and S-Aqueous/PVA samples ($p< 0.05$), but an insignificant difference ($p>0.05$) was observed in comparison to those of G7 (1.14 ± 0.03 and 2.54 ± 0.03 log CFU/g, respectively) in which the samples enclosed in S-AgNP/PVA-Pho films. Thus, The less effectiveness of the both nano-groups (S-AgNP/Ult-PVA, AgNP/PVA-Pho) on eliminating the *E. coli* (Table 3) was observed, which may be due to the resistance formation to AgNPs due to the production of flagellin, an adhesive protein of bacterial flagellum, resulted in aggregation of AgNPs (Kaweeteerawat et al., 2017) or due to the cell wall lipopolysaccharides (Alboofetileh et al., 2014), which in turn retarded their antibacterial efficiency. This result indicated that both S-AgNP/PVA films couldn't completely eradicate *E. coli* in fresh fish preserved in refrigerator.

3.1 Salmonella spp were not detected from the samples of whole groups (data was not included)

The Sensory properties of rainbow trout fillets coated with different nanocomposite films during refrigerated storage are presented in Table 4. The fish samples were considered acceptable, while their sensorial scores were less than 4 (moderate). The score for appearance, color, odor, texture, and general acceptability increased ($P <0.05$) over the cold storage during 14 days. The above-mentioned criteria of control samples and trout wrapped with Aqueous/PVA were considered 'unacceptable' from the 3rd day due to less efficiency of coatings, consequently fish deterioration and high activity of microbial spoilage throughout the storage, regardless of the temperature degree. Dislike to this study (Table 4) showed that the fish scores of all criteria were less than 4 (moderate) up to 7th day in the trout enfolded by S-AgNP/PVA-Ult and S-AgNP/PVA-pho ($p>0.05$), surprisingly the shelf life of trout fillets encased in Chitosan Coatings incorporated with *Mentha spicata* EO and zinc oxide nanoparticles extended up to 16d at refrigerated condition (Shahbazi & Shavisi, 2018a) in which the PBC significantly was less than MAL by 3 log CFU/g. However, the samples coated with S-AgNP/PVA-pho and S-AgNP/PVA-Ult composites had a longer shelf-life up to 7 days (Table 4), as compared to the control and S/Aqueous fish samples. Likewise, the fillets of mackerel (*Scomberomorus commerson*) wrapped with pomegranate peel extract along with gelatin (G)-polycaprolactone composite film caused the shelf life to be increased up to 6 days (Khodanazary, 2019). In contrast, the shelf life of sea bass slices was prolonged up to 12 days, while they wrapped with ZnO nanoparticles/basil leaf-EO loaded in LDPE compared to control (Arfat et al., 2015). Unexpectedly, off-flavor was not documented by the panelists up to 16 days of refrigerated-trout fillets enfolded by nanochitosan alone or incorporated with Na acetate while PBC was more than 6 log CFU/g on 6th day (Kamani et al., 2020). Curcumin- nanoemulsion (Khoshbouy Lahidjani et al., 2020), as a strong well-known antimicrobial combination, could not postpone the spoilage of trout fillet more than 5 days when they were preserved at refrigerated temperature.

Table 4. The effect of nano- extract types and temperature on sensory criteria for preserved trout fillets (n=3).

coating/Nano.P	Temp. °C	Days	Appear.	Mean Rank		Texture	G. Acceptability
				Color	Odor		
Control	4	1	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}
		3	2.33^{b}	2.33^{b}	2.33^{b}	2.33^{b}	2.33^{b}
		7	4.00^{c}	4.00^{c}	4.33^{c}	4.00^{c}	4.10^{c}
		14	5.00^{d}	5.00^{d}	5.00^{c}	5.00^{d}	5.00^{d}
	8	1	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}
		3	2.66^{b}	2.66^{b}	2.66^{b}	2.66^{b}	2.66^{b}
		7	4.33^{c}	4.33^{c}	4.66^{c}	4.33^{c}	4.41^{c}
		14	5.00^{c}	5.00^{c}	5.00^{c}	5.00^{c}	5.00^{c}
S-Aqueous	4	1	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}
		3	2.33^{b}	2.33^{b}	2.33^{b}	2.33^{b}	2.33^{b}
		7	4.00^{cd}	4.00^{cd}	4.00^{cd}	3.66^{c}	3.91^{bd}
		14	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}
	8	1	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}
		3	2.33^{b}	2.33^{b}	2.33^{b}	2.33^{b}	2.33^{b}
		7	4.00^{bcd}	4.00^{bcd}	4.00^{bcd}	4.00^{bcd}	4.00^{bcd}
		14	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}
Ultrasound	4	1	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}
		3	2.00^{b}	2.00^{b}	2.00^{b}	2.00^{b}	2.00^{b}
		7	3.33^{bcd}	3.33^{bc}	3.33^{bc}	3.00^{bc}	3.25^{bc}
		14	4.66^{cd}	4.66^{cd}	5.00^{cd}	4.66^{cd}	4.75^{cd}
	8	1	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}
		3	2.33^{b}	2.33^{b}	2.33^{b}	2.33^{b}	2.33^{b}
		7	3.33^{bc}	3.33^{bc}	3.33^{bc}	3.00^{b}	3.31^{bc}
		14	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}
Photo-chemical	4	1	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}
		3	1.66^{ab}	1.66^{ab}	1.66^{ab}	1.66^{ab}	1.66^{ab}
		7	3.00^{c}	3.00^{c}	3.00 ^{c}	3.00^{c}	3.00^{c}
		14	4.66^{d}	5.00^{d}	5.00^{d}	4.66^{d}	4.80^{d}
	8	1	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}
		3	2.00^{b}	2.00^{b}	2.00^{b}	2.00^{b}	2.00^{b}
		7	3.33^{c}	3.33^{c}	3.33^{c}	3.33^{c}	3.33^{c}
		14	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}	5.00^{d}

Extract/Nano.P: The production methods of *S. rechingeri* -Ag/NPs; Apear. = Appearance; Temp = Temperature, G. = General. Dissimilar superscripts show significant difference ($p<0.05$) in each column among 32 groups.

4 Conclusion

To conclude, the present study seeks to address using the green synthesis of AgNPs/*S. rechingeri*. The physical targeted criteria of AgNPs fabricated through ultrasound, and photochemical methods resulted in preferred properties, features, and desired nano-scale. The importance of these techniques is related to their antimicrobial efficiency and the environmentally friendly AgNPs-*S. rechingeri*/PVA synthesis. AgNP/Ult.PVA and S-AgNP/Pho.PVA films with size mostly smaller than 35-50 nm average diameter were effectively postponed the mesophilic and psychrophilic bacteria growth until the 7th day of cold storage period, while fish samples enclosed in *S. rechingeri*/PVA and control films could be edible up to 3rd day of cold storage due to their counts exceeded 6 log CFU/g. The bacteriological analyses were confirmed with the sensory evaluation so that *O. mykiss* samples enfolded with AgNP/Ult.PVA and S-AgNP/Pho.PVA films had desirable sensory criteria which made them be edible up to the 7th day even more. These two types of films were also capable to completely eliminate *S. aureus* from *O. mykiss* samples up to two weeks. The *E. coli* were less sensitive to AgNP/Ult.PVA and S-AgNP/Pho.PVA films compared to the other afore-mentioned bacteria through the cold storage. It suggested that AgNP/Ult. PVA and S-AgNP/Pho.PVA films can be used as a coating films for *O. mykiss* at cold preservation to prolong the shelf life up to 7th day in fish trails.

Acknowledgements

Technical support for the thesis accomplishment by the Islamic Azad University is gratefully acknowledged.

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