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Active Polyvinylpyrrolidone Enriched with Garlic Extract

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ABSTRACT

Introduction: Recently, there has been growing demand for the fabrication of edible antimicrobial and antioxidant compounds for the monitoring of foodborne bacterial diseases, which are mainly responsible for the contamination of raw foodstuffs. The present study aimed to use polyvinylpyrrolidone (PVP) enriched with garlic extract to control *Listeria monocytogenes* (LM) and *Staphylococcus aureus* (SA) in fresh sheep meat at over-chilled temperature for one week.
Methods: The obtained minced meat were divided into 100-gram portions, inoculated with SA and LM (7 log CFU/g), aseptically packed in designated PVP containing garlic extract (0%, 0.25%, and 0.5%), and transferred to a sterile polyethylene bag. All the samples were stored at refrigerated temperature for one week.
Results: PVP enriched with garlic extract at the concentrations of 0.25% and 0.5% could decrease the LM and SA counts to 2.30-2.82 and 2.51-1.36 log CFU/g, respectively (P<0.05).
Conclusion: According to the results, PVP containing garlic extract at the concentrations of 0.25% and 0.5% exerted inhibitory effects against LM and SA in raw sheep meat.

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Introduction

Recently, there has been growing demand for the fabrication of edible antimicrobial and antioxidant films for the monitoring of foodborne bacterial diseases, which are mainly responsible for the contamination of raw foodstuffs [1]. Polyvinylpyrrolidone (PVP) is a synthetic-based material, which is biodegradable and mostly applied in packaging, pharmaceutical, and cosmetic products. PVP breaks down in distilled water and is remarkably hygroscopic, making it impossible to be used as an active packaging material [2]. Furthermore, PVP has numerous biological properties, such as antiviral, antifungal, antioxidant, antibacterial, and antitumor effects [3].

According to the literature, composite and/or bilayer films supplemented with natural antimicrobial compounds are viable options to increase the quality and safety of perishable foodstuffs [4, 5]. Parsley, oregano, cinnamon, mint, and ziziphora are commonly used herbal essential oils and extracts as antimicrobial, antiviral, and antioxidant additives in biodegradable coatings/composite-based films [6-8].

Garlic extract has been shown to have remarkable inhibitory effects against the growth of gram-positive and gram-negative bacterial microorganisms, as well as notable antioxidant and antiradical properties [9]. Ancient Egyptians were aware of the attributes of garlic and benefitted from its medicinal and culinary purposes [10]. Garlic is a popular spice across the world, which could prevent and cure various diseases, such as respiratory tract infections, intestinal disorders, and cardiac problems [11]. Moreover, garlic is used as a natural antimicrobial/antioxidant preservative in active/smart food packaging [12]. It could be added to food packaging materials without additional processing technologies owing to its water solubility and physicochemical properties [13]. Garlic is predominantly cultured in Asia, Europe, and America [14]. To date, more than 100 compounds with antimicrobial and antioxidant properties have been isolated from garlic extract/essential oil [15].

Consumption of raw sheep meat and other red meats has been confirmed as a common source of foodborne pathogenic microorganisms, including *Salmonella enteritidis*, *Escherichia coli*

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O157:H7, *Bacillus cereus* (BC), *Bacillus subtilis* (BS), *Listeria monocytogenes* (LM), and *Staphylococcus aureus* (SA) [16, 17]. According to the Center for Disease Control and Prevention (CDC), approximately 48-50 million cases of foodborne diseases have been recorded in the United States, as well as 245,777 hospitalizations and 5,643 deaths that are associated with these diseases [18].

In recent decades, the consumption of ready-to-eat and ready-to-use meat has given rise to the increased outbreaks of foodborne diseases across throughout the world [18-20]. In addition, several outbreaks of foodborne illnesses have occurred due to the consumption of fresh food products such as fish and fishery products, meat and meat products, raw milk, and vegetable salads [18].

Raw sheep meat is consumed frequently in various regions in Iran. In general, the short shelf-life of unprocessed sheep meat stored at chilled temperatures is due to lipid and protein oxidation and microbial contamination [17]. Recent studies in this regard have emphasized on the importance of controlling various foodborne pathogens, especially LM and SA [21, 22].

The present study aimed to use PVP enriched with garlic extract to control LM and SA in raw sheep meat during refrigerated storage for one week.

Materials and Methods

Experimental Materials

In this study, PVP and all the solvents, chemicals, and microbial culture media were obtained from Merck, Germany. Garlic extract was purchased from Adonis Gol Darou in Tehran, Iran. In addition, SA (ATCC 6538) and LM (ATCC 19118) were obtained from the microbial archive of Faculty of Veterinary Medicine at Razi University in Kermanshah, Iran.

Preparation of Active PVP

Two grams of PVP was dissolved in 20 milliliters of distilled water and mixed for three hours at an ambient temperature under magnetic stirring (IKA, Germany). Garlic extract at the concentrations of 0%, 0.25%, and 0.5% was added to the solution and stirred for one hour. Afterwards, glycerol (0.5% v/v) was added to the solutions. All the solutions were poured using the casting method into approximately 50 milliliters of the mixed solutions on 12-centimeter glass casting plates and dried at room temperature for 72 hours.

Preparation of Microorganisms

Each pathogenic strain was selected from a single colony on the Brain Heart Infusion (BHI) agar, cultured at the temperature of 35±1°C for 72 hours, and sub-cultured (0.1 ml) at the temperature of 35±1°C for 72 hours in 10 milliliters of the BHI broth. The density of the required microbial suspensions for antibacterial tests was investigated using a spectrophotometer at 632 nanometers. In addition, the detection of the inoculum dose (10^7 CFU/ml) was evaluated based on the plate count on the BHI agar [23].

In-vitro Antimicrobial Effects of the Prepared Active PVP

The *in-vitro* antimicrobial effects of the prepared active PVP were evaluated using the broth microdilution assay [24]. In this process, aseptic microdilution plates with U-bottom wells were used by dispensing 180 microliters of PVP supplemented with various concentrations of the garlic extract (0%, 0.25%, and 0.5%) and 20 microliters of the bacterial cultures at the inoculum dose of 1×10^7 CFU/ml of the investigated bacteria. The last microplate well containing 180 microliters of the BHI broth without the antimicrobial compound and 20 microliters of the bacterial inoculum was considered as the positive group. Following that, the plates were packed with clean plate sealer. The microplate was shaken using a plate shaker for one minute and incubated at the temperature of 35°C overnight.

At the next stage, sampling was performed for each microplate well using 10-fold serial dilutions with the BHI broth, followed by culturing on the BHI agar and incubation for at the temperature of 35°C for 48 hours. After incubation, the bacterial colonies were enumerated, and the obtained data were expressed in terms of the differences in the bacterial population (DP) using the following equation:

$$Log\ DP = log\left(\frac{N}{N^0}\right) = log\ N - log\ N_0$$

Where N and N_0 represent the bacterial population (CFU/ml) at times t and zero, respectively.

In-vitro Antioxidant Effects of the Prepared Active PVP

The *in-vitro* antioxidant effects of the prepared active PVP evaluated using the free radical scavenging activity of 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) [24]. To do so, 25 milligrams of the designated PVP was incorporated into three milliliters of distilled water, and 2.8 milliliters of the film extract solution was added, along with 0.2 milliliter of 1 mM methanolic DPPH solution. Afterwards, the solution was shaken and maintained at an ambient temperature in the dark for 30 minutes, and the absorbance was determined at 517 nanometers. The percentage of DPPH radical scavenging activity was also determined using the following equation:

$$DPPH\ Scavenging\ Effect\ (\%) = \frac{[(Abs\ DPPH - Abs\ film\ extract)]}{Abs\ DPPH} \times 100$$

Preparation of Raw Sheep Meat

Thigh muscle meat was purchased from a local butchery Kermanshah, Iran. The meat samples were immediately placed in sterile stomacher bags and transferred to the laboratory. The outer surface of the samples was removed, sprayed with ethanol (95% v/v), and burnt. Following that, the burnt sections were trimmed to reduce microbial surface contamination. The meat cubes were ground using a sterile steel meat grinder (Parskhazar, Tehran, Iran) and autoclaved at the temperature of 121°C for 20 minutes. Afterwards, the obtained samples were divided into 100-gram portions, contaminated with SA and LM, aseptically packed in the designated PVP, and placed in a sterile polyethylene bag. The minced meat samples were preserved at chilled temperature for one week.

Microbiological Analysis of Minced Sheep Meat

For plating, 10 grams of each sample was maintained in sterile conditions inside clean stomacher bags containing 90 millilitres of 0.1% sterile buffered peptone water and mixed in a stomacher (Interscience, France). Plating was conducted on the PALCAM Listeria Selective Agar (LM) and Baird-Parker agar (SA), and the samples were incubated at the temperature of 37ºC for 24 hours. The obtained data were expressed in log CFU/g, and all the experiments were performed in triplicate.

Sensory Evaluation of Minced Sheep Meat

To determine the effects of the designated packaging on the sensory properties of the uninoculated minced meat, nine panelists aged 22-32 years (three males and six females) examined the meat samples in terms of the odor and color based on a nine-point hedonic scale (Extremely Dislike=1, Neither Like nor Dislike=5, Extremely Like=9) [25]. The samples were labeled with random three-digit numbers, placed in small white plastic glasses, and served immediately.

Results and Discussion

Table 1 shows the findings regarding the antimicrobial activities of the designated PVP containing the garlic extract (0%, 0.25%, and 0.5%). Accordingly, the designated PVP supplemented with the garlic extract effectively decreased the LM and SA counts as evidenced by -1.45--2.09 and -1.23--1.87 log CFU/ml of DP, respectively. In a similar research, Daka et al. (2011) [26] reported that garlic extract had great antibacterial activity against SA *in-vitro*. Furthermore, Teixeira et al. (2014) [13] claimed that fish protein film incorporated with garlic extract exerted inhibitory effects against LM based on the broth microdilution assay.

Table 1. *In-vitro* Antibacterial Activity (log DP*) of Polyvinylpyrrolidone (PVP) Enriched with Garlic Extract

	Staphylococcus aureus	*Listeria monocytogenes*
PVP	ND	ND
PVP + 0.25% Garlic Extract	-1.23±0.09	-1.45±0.12
PVP + 0.5% Garlic Extract	-1.87±0.81	-2.09±0.25

**Log DP= log (N/N_0) = log N – log N_0; where N and N_0 are bacterial population at times t and zero (10^5 CFU/ml), respectively; ND=not determined.*

According to the results of the present study (Table 2), the DPPH radical scavenging activity of the PVP supplemented with 0.25% and 0.5% garlic extract was 22.34% and 36.57%, respectively. To date, no articles have been published regarding the antioxidant properties of PVP combined with garlic extract. In a study in this regard, Lawrence et al. (2011) [27] reported

that the DPPH and nitric oxide scavenging assays of garlic extract were 0.5 mg/ml and 50 µg/ml, respectively. In addition, the findings of Lu et al. (2017) [28] indicated that the DPPH radical scavenging activity of black garlic extract was 5-70 µg/ml. On the same note, Toledano Medina et al. [29] stated that the antioxidant capacity of black garlic whole bulbs was within the range of 16-109 µg/ml. Furthermore, Bozin et al. (2008) [30] elaborated on the mechanism of the DPPH assay, denoting that garlic extract could act as the donor of hydrogen/electrons in the reduction of the DPPH radical into the reduced form of DPPH. During the experiment in the mentioned study, the IC_{50} value was estimated at 1.03 mg/ml for immature garlic extract, 4.41 mg/ml for air-dried garlic bulb extract, and 6.01 mg/ml for fresh garlic extract.

Table 2. Antioxidant Properties of PVP Enriched with Garlic Extract

	DPPH%
PVP	ND
PVP + 0.25% Garlic Extract	22.34±3.18
PVP + 0.5% Garlic Extract	36.57±4.50

ND=not determined.

Figures 1-2 depict our findings regarding the effects of the prepared PVP containing the garlic extract (0%, 0.25%, and 0.5%) against LM and SA in the raw meat samples, respectively. As can be seen, both pathogenic microorganisms could survive at the refrigerated temperature during one week. This is consistent with the previous findings reported for minced trout fillet [31], fresh chicken fillets [25], fresh salmon [32], and chicken meat products [33].

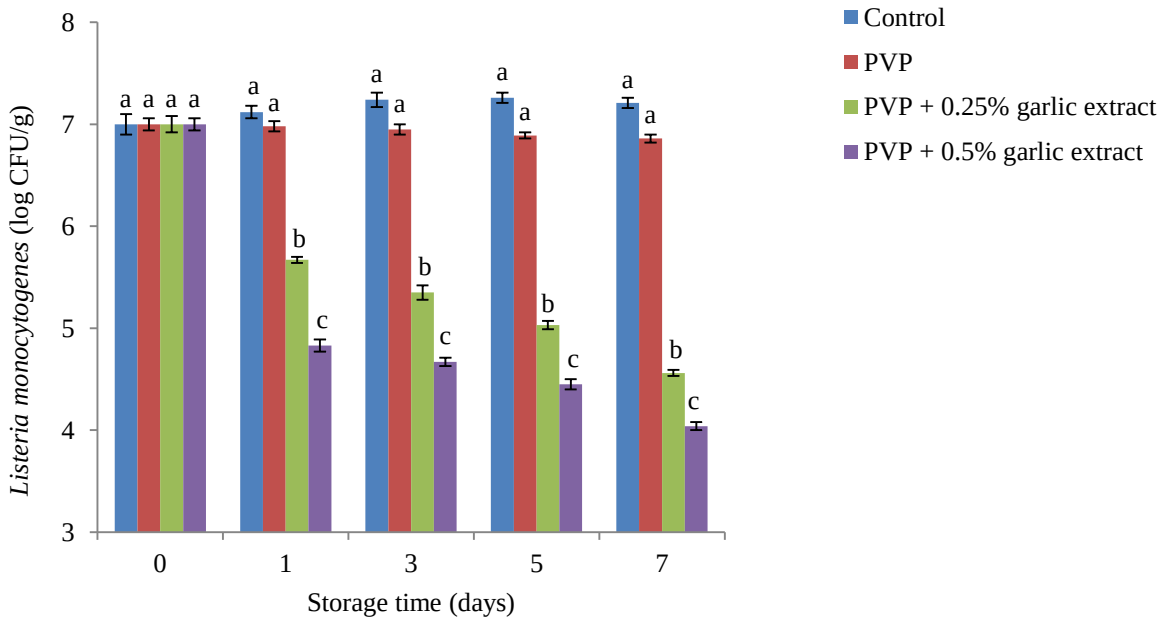

Figure 1. Antibacterial activity of polyvinylpyrrolidone (PVP) enriched with garlic extract against *Listeria monocytogenes* in raw sheep meat. Different lower case letter indicates significant differences among groups ($P < 0.05$).

According to the findings of the current research, pure PVP had no antimicrobial properties against LM and SA, and the final counts reached 6.86 and 5.87 log CFU/g, respectively. This finding is in line with the previous *in-vitro* results regarding antimicrobial activity (Table 1). Moreover, PVP enriched with 0.5% garlic extract was observed to reduce the counts of LM and SA up to 2.82 and 1.36 log CFU/g, respectively ($P<0.05$).

Several studies have demonstrated that herbal essential oils/extracts could significantly suppress the growth of bacterial pathogens, especially LM and SA. For instance, Zhang et al. (2016) [34] concluded that the major compounds found in herbal essential oils/extract could effectively destroy the bacterial cytoplasmic membrane, which provides permeability inhibition to critical ions such as K^+, Na^+, and H^+; these ions are essential to the

extension of cell membrane functions and storage of the functional attributes of enzymes for their normal metabolism. In addition, impermeability to critical ions is sustained and managed by the structure and chemical constituents of its membrane.

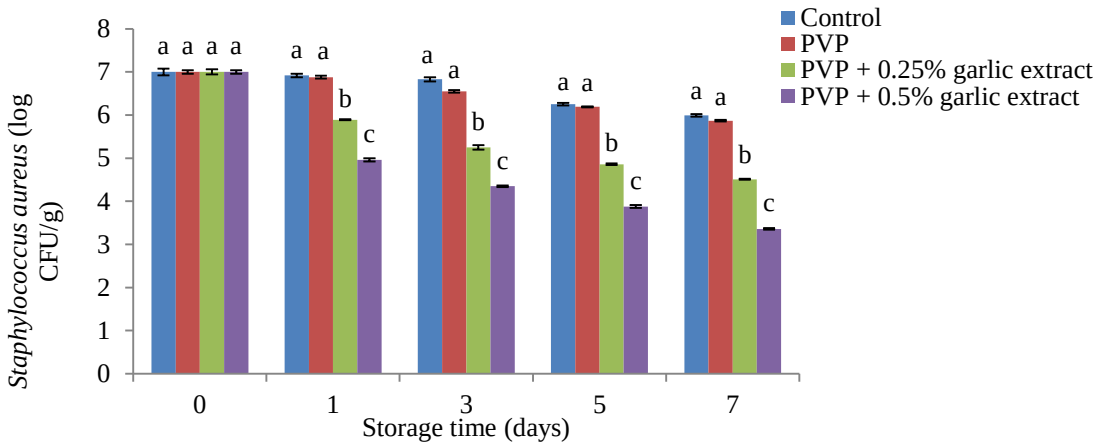

Figure 2. Antibacterial activity of polyvinylpyrrolidone (PVP) enriched with garlic extract against *Staphylococcus aureus* in raw sheep meat. Different lower case letter indicates significant differences among groups (P < 0.05).

Maintaining ion homeostasis is considered essential to the energy balance status of cells as it plays a pivotal role in energy-related processes, including solute transport, metabolic control, management of turgor pressure, and motility. Therefore, even relatively minor variations in the structure of membranes might lead to complications in the cell metabolism and cause microbial cell death [18].

In another research, Shahbazi (2017) [24] reported that chitosan and gelatin films incorporated with red grape seed extract and *Ziziphora clinopodioides* essential oil exerted inhibitory effects against *S. typhimurium*, *E. coli* O157:H7, BC, BS, LM, and SA. On the other hand, Kristo et al. (2008) [35] reported that sodium caseinate supplemented with sodium lactate, potassium sorbate, and nisin could decrease the growth of LM more significantly compared to the untreated group. In another study [36], alginate-based films containing oregano, cinnamon, and savory essential oils were reported to reduce inoculated LM and *S. typhimurium* in bologna and ham from 5 log CFU/g to the detection level (<1 log CFU/g) after five days of refrigerated storage. Figure 3 shows the sensory properties of the raw sheep meat, such as odor and color. As is observed, 0.5% garlic extract negatively affected the odor of the treated samples. Moreover, the control group had the lowest sensory scores. The optimal results in this regard were obtained in the PVP supplemented with 0.25% garlic extract. Several studies have investigated the effects of additive natural compounds on fresh and processed foods in order to predict the applicability of food products in terms of consumer acceptance [25, 37, 38], and their findings are generally consistent with the results of the present study.

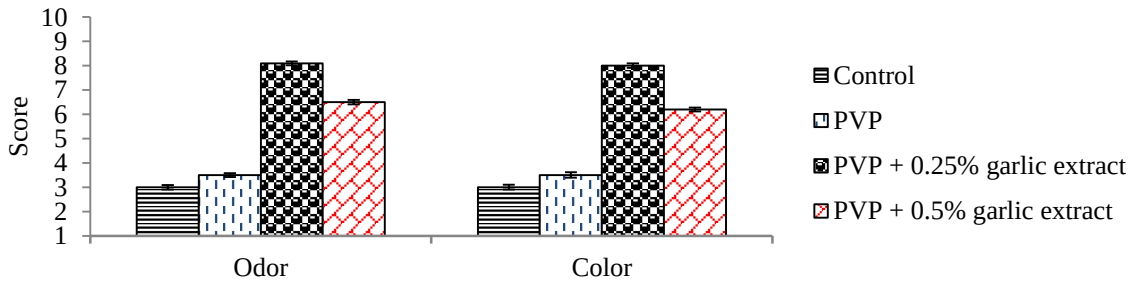

Figure 3. Sensory Properties of Uninoculated Raw Sheep Meat

Conclusion

Fabrication of PVP containing various concentrations of garlic extract was successfully achieved in the present study. Furthermore, satisfactory results were obtained *in-vitro* regarding the antimicrobial and antioxidant properties of the designated materials, which could help researchers to recognize their activity in various food models (e.g., raw sheep meat). In our food model, the prepared materials could remarkably suppress the bacterial growth of LM and SA within one week.

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