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Scientific Research

Effects of Poly Lactic Acid Film Containing Sumac Fruit Extract and Copper Oxide Nanoparticles on Shelf Life Enhancement of Minced Beef

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ABSTRACT

Shelf life increasing of red meat has the great importance due to its perishable properties and consumers' demand to use food free synthetic preservatives. This study was aimed to investigate the antimicrobial and antioxidant effects of poly lactic acid film (PLA) containing different concentrations of ethanolic sumac extract (SE 1 and 3%) and copper oxide nanoparticles (CuO1 and 2%) on shelf life enhancement of the minced beef during cold storage. The samples were stored at 4 ± 1 °C for 12 days and analyzed at 0, 1, 3, 6, 9 and 12 days of the storage period. All of the treatments significantly ($P \leq 0.05$) decreased total viable count, psychrotrophic bacteria and *Enterobacteriaceae* spp. population as compared control group during the storage period. Total volatile basic nitrogen (TVB-N) and pH values of the non-packed samples (control) were significantly ($P \leq 0.05$) higher than the packed ones during the storage time. The combined treatments containing SE and CuO-NPs showed the highest efficiency in shelf life increasing of the cold stored minced beef in dose dependent manner. Based on the results, it can be concluded that the usage of biodegradable poly lactic acid film containing a complex of SE and CuO-NPs is effective packaging in the shelf life improvement of minced beef during 12 days of the refrigerated storage period.

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1- Introduction

Red meat is the richest source of protein for human nutrition [1]. Minced red meat is one of the meat products, which is actually red meat that is usually prepared from parts of the carcass that have more fat and are less tender and crispy. Minced red meat is classified as one of the types of meat products that are very sensitive to spoilage, which can be kept for a short time under special conditions and must be carefully protected [2]. This product has a high potential for contamination due to the different stages of production as well as increasing the contact level of meat with air and microorganisms. Also, this type of meat provides a suitable environment for the growth of microorganisms and spoilage due to its permeability to oxygen due to its porous texture, high water activity and its richness in nutrients [3]. Many factors such as storage temperature, atmospheric oxygen, light, microorganisms and internal enzymes are involved in meat spoilage [4]. Various methods are used to preserve meat and meat products, including heating, drying, freezing, irradiation, adding salt, adding antimicrobial substances, and some of these methods leave problems in fresh meat from different aspects, which can cause damage to the quality of the product. meat or become a threat to the health of the consumer. Therefore, producers are looking for methods that increase the shelf life of the product and are economical for them. Therefore, the use of active packaging containing antimicrobial substances can be a suitable method to preserve meat from external and internal factors [6 and 5].

Polylactic acid polymers are one of the most common polymers used in packaging, which are produced from lactic acid monomers resulting from fermentation, which are mainly L-lactic acid [7]. This biodegradable polymer, derived from lactic acid, is a substance with many capabilities, which is made from 100% renewable resources such as corn, sugar beet, wheat and other products rich in sugar, it is non-volatile and odorless, and is one of the materialsGRAS are classified [8]. This polymer is transparent and has the properties of inhibiting the release of aroma, taste, moisture and fat from the food, inhibition against the passage of ultraviolet light and high mechanical strength [9]. In recent years, antimicrobial films based on polylactic acid have been designed using natural and synthetic antimicrobial compounds.

Due to the unique feature of plant extracts and volatile oil as a source of natural antimicrobial compounds, the use of natural resources, including plants that grow wild, for the preparation of antimicrobial plant extracts in foods, has gained great importance [10].]. Sumac with scientific name(*Rhus coriaria* L.) from familyCashew nutsIt is a shrub from the pistachio family, which is 1-5 meters high and has compound leaves that contain 9-15 leaflets covered with fluff and seeds. The ripe fruits of this plant are red to brown. Sumac is a plant that grows wild in large areas of the Canary Islands, on the coasts of the Mediterranean, Iran and Afghanistan, and natively in areas of southeastern Turkey. This plant has a bitter and acidic taste and contains flavonoids, phenolic acid, citric acid and tartaric acid [11].

Metal nanoparticles are other antimicrobial agents that have received more attention in recent years. Nanoparticles have good antimicrobial activity due to features such as small particle size and as a result of increasing surface-to-volume ratio and high reactive activity [12]. Metal nanoparticles can be attached to the membrane of microorganisms through electrostatic reaction and cause cell death by interfering with the transport of nutrients [2]. Metal

nanoparticles of copper oxide cause destruction in bacteria by direct effect on bacterial membrane. This effect is the result of the reaction of copper ion with amine and carboxyl groups of the peptidoglycan layer of the bacterial wall and as a result of damage to the cell wall and bacterial membrane. Environmental conditions, concentration of copper ions and the type of microorganism affect the antimicrobial power of copper against microorganisms [13].

Extensive studies have been conducted in relation to the direct use of active antimicrobial compounds in food, but the tendency to use this type of material indirectly in the form of food packaging is increasing day by day. Therefore, according to this topic, the aim of this research is to design and produce active and biodegradable films based on polylactic acid containing different concentrations of sumac ethanolic extract and nano copper oxide by molding method and to investigate the antimicrobial performance of the films in the packaging of minced meat at 4 degrees Celsius. Is.

2- Materials and methods

2-1- Raw materials

Polylactic acid granular polymer, which was prepared as a polymer substrate in the form of white granules with a density of 1.3 grams per cubic centimeter and a molecular weight of 197,000 daltons, was obtained from Merck, Germany. Copper oxide nanoparticles were purchased from the agency of Sigma (Germany). Nutrient Agar, Violet Red Bile Agar, Plate Count Agar Peptone water, Tween 80, ethanol and chloroform were purchased from Merck (Germany) and sumac fruit was purchased from Hamadan market.

2-2- Sumac ethanol extract extraction

250 grams of red sumac fruit powder was added to the mixture of ethanol and sterile distilled water (in a ratio of 7:3) and the mixture was filtered through Whatman No. 40 paper after homogenization overnight. Finally, the filtered solution was concentrated in a rotary and the final concentrate was stored at 4 degrees Celsius until the experiment.

2-3- Preparation of polylactic acid films containing sumac extract and copper oxide nanoparticles

Polylactic acid films and their nanocomposites were prepared using solvent casting method. To prepare polylactic acid film, 1 gram of polylactic acid was dissolved in 50 ml of chloroform and stirred for 8 hours at room temperature. Then Tween 80 at the rate of 0.25% solution as an emulsifier and also according to the type of treatment, the desired concentrations of copper oxide nanoparticles (1 and 2% w/w) and sumac ethanol extract (1 and 3% w/w) were added to the solution. Specific films were added to each treatment and well homogenized by homogenizer (12000 rpm). The film-forming solution was poured on glass molds with a diameter of 12 cm and after drying, it was used to cover the minced meat.

4-2- Preparation and packaging of minced meat with antimicrobial films

Fresh cold veal meat (after mortuary freeze) was transferred to the food laboratory of the Faculty of Paraveterinary Medicine of Bo Ali Sina University in completely sterile and cold conditions. It was immediately cut into pieces under sterile conditions and ground by a sterile meat grinder and packed in polylactic acid films containing different concentrations of sumac extract and copper oxide nanoparticles. Finally, they were stored in sterile polyethylene bags for 12 days at intervals of days 0, 1, 3, 6, 9, and 12

to perform relevant tests under refrigerator conditions.

2-5- Microbial analysis of samples

To check the antimicrobial effect of the films, 10 grams were taken from each sample and homogenized with 90 ml of 0.1% peptone water dilution solution. Then, serial dilutions were prepared and cultured in the desired culture media. Microbial culture of the samples for total counting of aerobic bacteria (in culture medium of Kant agar plate), spirochetes (in culture medium of Kant agar plate) and bacteria of the Enterobacteriaceae family (in culture medium of violet red bile agar) at 6 different times including days 0 (beginning of the study)), 1, 3, 6, 9 and 12 (the end of the study). Also, in this study, the sample without polylactic acid film (only minced meat) was considered as the control group.

6-2- Chemical analysis of samples

Chemical investigation of total volatile base nitrogen(TVB- N) Meat samples treated according to the methodAOAC It was done in three replicates for each sample. to measurepH10 grams of minced meat was added to 90 ml of distilled water and homogenized for 5 minutes by a stirrer, then the obtained mixture was filtered by Whatman filter paper andpH The gauge was measured in three repetitions.

2-7- Statistical analysis

The analysis of the results for each sample was done as follows: All the experiments were done in the form of a random design and in at least two repetitions. After performing various tests on samples containing polylactic acid film containing different concentrations of sumac ethanolic extract and copper oxide nanoparticles, the data were recorded in Excel software. For the statistical analysis of the data of the microbial and chemical section, the effect of the films on the food sample from the softwareSPSS Version 20 was used and amountP Less than 0.05 was considered statistically significant. Analysis of variance test was used to check the significant difference between treatment and control conditions. Duncan's statistical test was used to compare the difference between means at the 0.05 level.

3. Results and Discussion

1-3- Microbial analysis

The results of total counting of aerobic bacteria, cold-loving bacteria and Enterobacteriaceae family bacteria in minced meat samples packed with polylactic acid active film containing sumac ethanolic extract and copper oxide nanoparticles during storage at 4 degrees Celsius for 12 days in figures 1 to 3 is shown.

The results of this study showed that the number of total bacteria in all the different treatments investigated in this study increased with the passage of time and the number of mentioned bacteria in the samples packed with polylactic acid film containing different concentrations of sumac ethanolic extract and copper oxide nanoparticles.

compared to the control group significantly (>0.05).P) was less. Also, among the different films, the highest antimicrobial effect was related to the polylactic acid film containing 3% sumac extract and 2% nano copper oxide.

On the zero day of the study, the total count of bacteria in the control groublog CFU/g It was 3/92. The highest limit for total bacterial count in quality meatCFU/g log 7 is [14]. In this study, the total number of bacteria for the control group at the end of the maintenance period (day 12) waslog CFU/g Received 9/14 (Figure 1). In this group, on the 6th day of the study, the number of bacteria was approxCFU/g log 7 receipts. Also, on different days, the total count of samples packed with films containing sumac ethanolic extract and copper oxide nanoparticles was significantly (>0.05)P) was lower than the samples of the control group, which may be due to the activation of the antimicrobial properties of the agents used in the films, whose effectiveness depends on the concentration and type of the antimicrobial substance, which is consistent with previous works [15]. It should be noted that in the samples that were packed with polylactic acid film alone, compared to the group without polylactic acid coating, a decrease in microbial growth was seen, which could be due to the low permeability of polylactic acid film to oxygen, which is in agreement with the results of previous studies. corresponds to [16]. The results of this study showed that among different films, the best effect was related to the film containing sumac ethanol extract 3% and copper oxide nanoparticles 2%. The least antimicrobial property in preventing and reducing the growth of mesophilic bacteria was observed in the groups treated with polylactic film alone. In total, the statistical analysis of variance investigated the effect of different concentrations of sumac extract and copper oxide nanoparticles separately and in combination on the dependent variable (the logarithm of the number of aerobic mesophilic bacteria inCFU/g) showed that there is a significant difference among the different studied groups (<0.05).P).

In Figure 2, the results of counting gram-negative cold-oriented bacteria as the most important group of effective bacteria in the creation and development of microbial spoilage in meat products and meat products in meat samples packed with polylactic acid films containing different concentrations of sumac ethanol extract and Nano copper oxide has been reported. Cryogenic bacteria are the most important group of microorganisms of microbial contamination in fresh meat under cold storage conditions [17]. The obtained results showed that the number of cold-oriented bacteria increased significantly during the storage period with the passage of time. The highest increase in the number of cold-oriented bacteria was observed in the control sample. On the zero day of the study, the total count of bacteria in the control groublog CFU/g It was 3/51 that at the end of the maintenance period (12th day) tolog CFU/g Received 8/95 (Figure 2). Also, on different days, the total count of samples packed with films containing sumac ethanolic extract and copper oxide nanoparticles was significantly (>0.05)P) was less than samples not wrapped with film or wrapped with film without ethanol extract and copper oxide nanoparticles.

Bacteria of the Enterobacteriaceae family as indicator microorganisms also form a part of the microflora of veal meat, therefore, it is necessary to study their growth process while keeping veal meat at refrigerator temperature. The reasons for the growth of Enterobacteriaceae bacteria in cold conditions are unknown [18]. The results related to the effect of the studied films are reported in Figure 3. According to the results of the present

study, the number of Enterobacteriaceae bacteria in the control group waslog CFU/g 2.75 on the day of Safar tolog CFU/g 7/12 arrived on the 12th day. While in the samples containing 3% sumac extract and 2% copper oxide nanoparticles on the 6th day of the study, the number of bacteria of this familylog CFU/g It was 3/01. It should be mentioned that in the groups that used a combination of extracts and nanoparticles, the growth rate of Enterobacteriaceae bacteria at the end of the 12th day of sample storage was approximately 1.83-3.3 logarithms compared to the control group. Was. According to previous studies, extracts have an effective role in reducing the number of Enterobacteriaceae bacteria in meat and meat products in cold conditions [19].

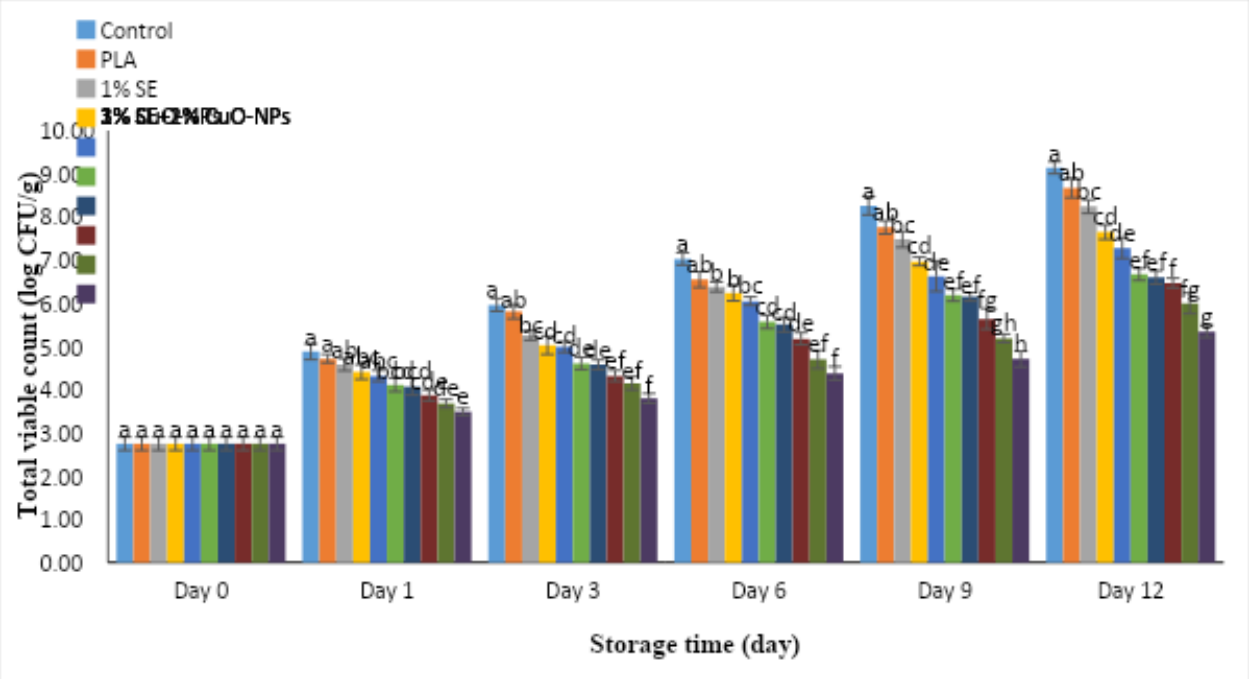

Fig 1 Changes in total viable count of the wrapped minced beef during refrigeration storage.

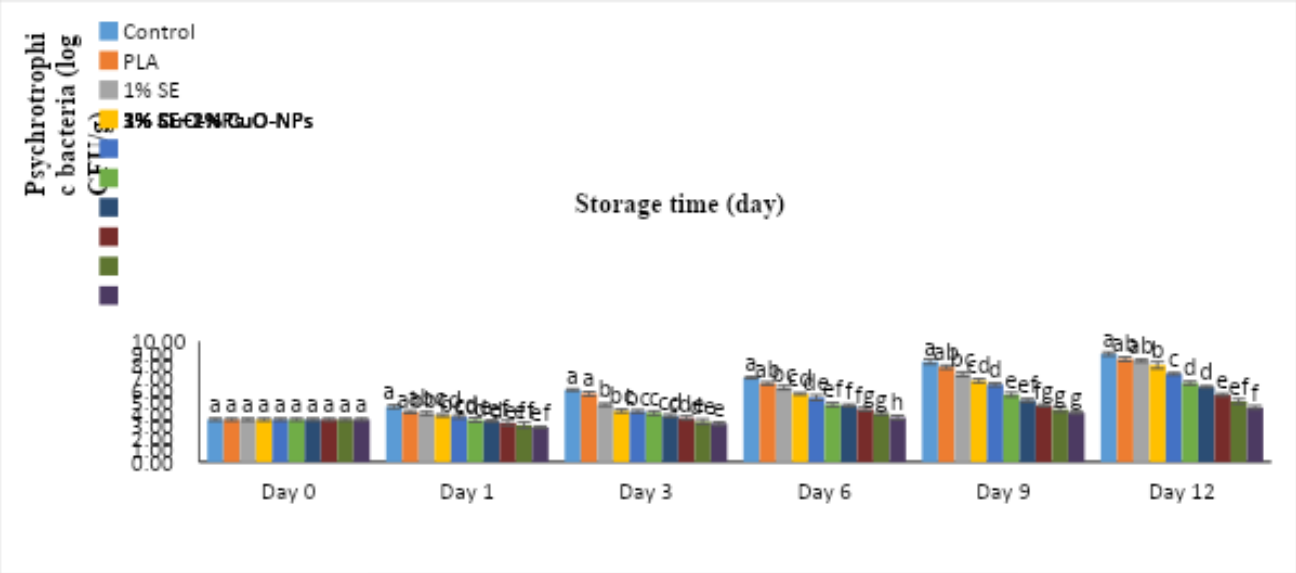

Fig 2 Changes in psychrotrophic bacteria of the wrapped minced beef during refrigeration storage.

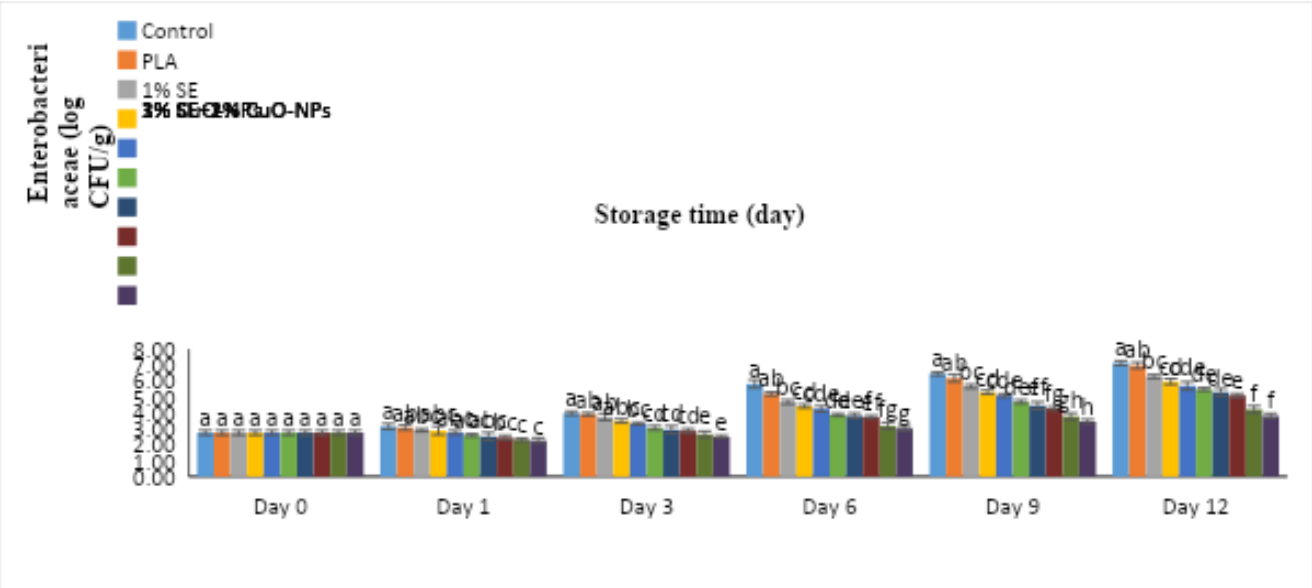

Fig 3 Changes in enterobacteriaceae of the wrapped minced beef during refrigeration storage.

2-3- Chemical analysis results

The measurement of volatile nitrogen substances is a quantitative parameter for determining the amount of ammonia and amines of the first, second and third type in red meat. An increase in this number indicates an increase in the activity of spoilage bacteria and meat enzymes. Changes in the amount of volatile nitrogen substances in beef samples are shown in Figure 4. The initial amountTVB-N In the samples, it was 10.43 milligrams per 100 grams of meat, which reached 45.87

milligrams per 100 grams of meat at the end of the 12th day of keeping the samples. By increasing the maintenance period, the amountTVB-N It increased in all treatments, but in the samples that were packed with films containing extracts and nanoparticles, this increase was less compared to the control sample, which is in accordance with previous findings [19]. ChangespH In minced meat samples packed with different films during the storage period, it is reported in Figure 5. LevelpH The initial veal meat was 5.67, which reached 6.70 at the end of the study (day 12). the amount ofpH The samples were increasing from day zero (the first day of the study) to day 12 (the final day of the study), but this increase was less in the samples treated with sumac extract and copper oxide nanoparticles compared to the control sample. According to the report of Codex Organization in 2003,pH Animal meat should be 5.7-7 to be safe for consumers according to the codex. As the results of this research showed, changespH During the storage time, there was an increasing trend, and these changes are mainly due to the microbial activities and enzymatic activities of the meat during the storage period. Over time, meat tissue is destroyed by the enzymatic activity of meat microorganisms, which leads to the production of nitrogenous compounds in meat [20]. Changing ProcedurepH It has a direct relationship with the process of changes in the microbial population of the samples; That is, with the increase of the microbial population and as a result of the enzyme activity,pH also increases. The results obtained from the changespH It was consistent with the results of the total microbial population test; In this way, the packaged samples have a slower increase due to the lower microbial populationpH They had control over the sample. ComparisonpH The results obtained in this research with the results reported by other researchers showed that in some casespH Meat decreases at the beginning of the period and then increases in all cases. The increase of lactic acid bacteria and as a result the accumulation of lactic acid and then the increase of spoilage bacteria that

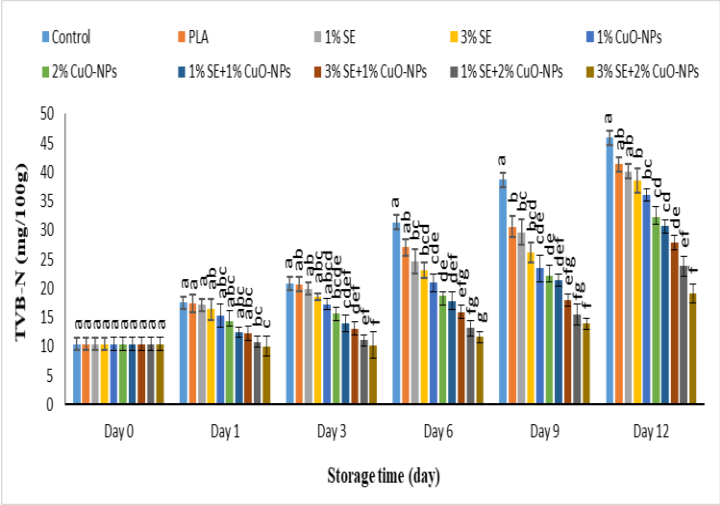

Fig 4 Changes in TVB-N of the wrapped minced beef during refrigeration storage.

lead to the accumulation of alkaline compounds such as ammonium and trimethylamine can be the cause of this [21 and 22].

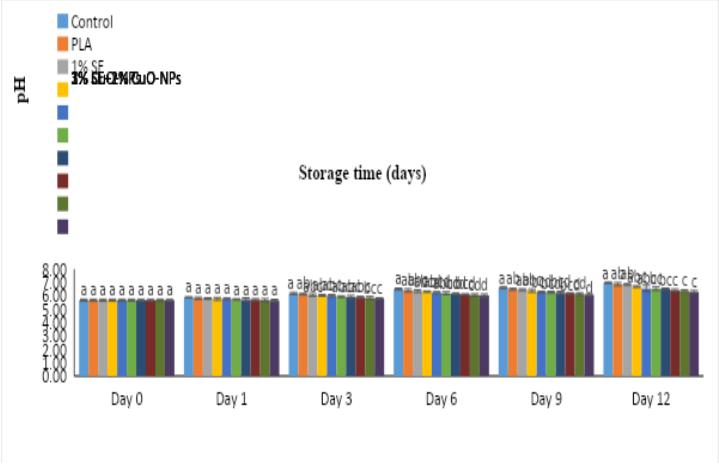

Fig 5 Changes in pH of the wrapped minced beef during refrigeration storage.

4- General conclusion

In the present study, based on the findings, it can be concluded that polylactic acid films containing sumac ethanolic extract and copper oxide nanoparticles alone and in a proper combination together improve the shelf life of minced veal compared to the control group during storage for 12 days. Storage in cold conditions has increased. Therefore, the use of this formulation can be considered a suitable method for preserving meat. Based on the obtained results, it can be concluded that polylactic acid film containing 3% sumac ethanolic extract and 2% copper oxide nanoparticles is the most effective film for increasing the shelf life of meat food samples.

5- Thanks

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6- Resources

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۲- دانش آموخته کارشناسی ارشد گروه بهداشت و کنترل کیفی مواد غذایی دانشگاه بوعلی سینا همدان.

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چکیده

افزایش ماندگاری گوشت قرمز بدلیل ماهیت فسادپذیر آن و تمایل مصرف کنندگان به استفاده از محصولات غذایی فاقد نگهدارنده از اهمیت بالایی برخوردار است. این مطالعه با هدف بررسی اثر ضدمیکروبی و آنتی اکسیدانی فیلم پلی‌لاکتیک اسید (PLA) حاوی غلظت‌های مختلف عصاره اتانولی سماق (SE ۱ و ۳ ٪) و نانو ذرات اکسید مس (CuO-NPs ۱ و ۲٪)، در افزایش ماندگاری گوشت چرخ شده گوساله طی مدت نگهداری در شرایط سرد صورت پذیرفت. نمونه ها در دمای۱± ۴ درجه سانتیگراد به مدت ۱۲ روز ذخیره شدند و در روزهای ۰، ۱، ۳، ۶، ۹ و ۱۲ مورد ارزیابی قرار گرفتند. نتایج این مطالعه نشان داد که جمعیت کلی باکتری‌های هوازی، سرماگراها و انتروباکتریاسه در نمونه‌های بسته‌بندی شده با فیلم‌های حاوی عصاره سماق و نانوذارت مس در مدت نگهداری، به صورت معنی‌داری (P<۰/۰۵) نسبت به گروه کنترل کمتر بود. مقادیر کل بازهای نیتروژنی فرار (TVB-N) و pH در نمونه‌های کنترل بالاتر از نمونه‌های بسته‌بندی شده حاوی ترکیبات مورد مطالعه بود. تیمارهای ترکیبی حاوی عصاره سماق و نانوذرات اکسید مس بالاترین کارایی را در افزایش ماندگاری گوشت چرخ شده در طول دوره نگهداری بصورت وابسته به دوز از خود نشان دادند. بر اساس نتایج به دست آمده می توان نتیجه گرفت که استفاده از فیلم زیست تخریب پذیر پلی‌لاکتیک اسید حاوی مخلوط عصاره اتانولی سماق و نانو ذرات اکسید مس در افزایش مدت زمان ماندگاری گوشت چرخ شده گوساله در طی ۱۲ روز نگهداری در دمای یخچال موثر بوده است.