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Packaging based on Ag-Low Density Polyethylene for shelf-life extension of pasteurized and traditional butters at refrigerated temperature

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Abstract

This article focuses on increasing the shelf life of both pasteurized (Pa) and traditional (Tr) butters wrapped in Ag-Low Density Polyethylene (LDPE) film in concentration of 2.5%, 7.5%, 12.5%, and 17.5% at refrigerated temperature up to one month. Silver nanoparticles were synthesized (20.63 nm) through the chemical reduction followed by the melt mixing method to produce Ag-LDPE films. A mixed model-ANOVA Repeated Measurement presented that total bacteria, *S. aureus* and *E. coli* were eliminated from the Pasteurized butter samples wrapped with 17.5% Ag/LDPE films. In comparison, psychrophilic bacteria can be eradicated by 2.5-17.5% Ag/LDPE films after 30d. Peroxide value showed a slight fall from 0.50 and 0.28 meq/kg, respectively for Pa and Tr butters on the 1st day in the control group to 0.31 and 0.24 meq/kg, respectively in the butter wrapped with 17.5% Ag-LDPE film at the end of the storage with no significant difference ($p > 0.05$) with other treatments. Iodine value was decreased after 30d. It is concluded that the use of 17.5% Ag/LDPE as a coating of butter can safely preserve pasteurized butter at least a month.

Keywords: butter; nano silver; chemical reduction; melt mixing; LDPE packaging.

Practical Application: Extending shelf life of butter using nanoparticles.

1 Introduction

Butter is a water-in-oil (W/O) emulsion produced by the inversion phase of cream, in which its proteins are the emulsifiers. Butter remains a stable compacted commodity when chilled (Schäffer et al., 2001) but must be liquid in blood for it to be safe for public health. Butter is one of the largest milk by-products. It is full rich in fatty acids and produced traditionally or industrially. Rancidity is a critical concern in butter preservation (Asdagh & Pirsa, 2020).

Fluorescent illumination, temperature alteration, or sunshine result in the oxidative changes of fat and microbial spoilage in butter. Lipolytic enzymes could be excreted from psychrotrophic, *Escherichia coli,* and positive coagulase *Staphylococcus aureus,* which have spoilage impact on off-flavors in butter, and other dairy products that decrease the shelf life and the products become unhealthy and unfavorable for consumption (Tola et al., 2019).

In food safety disciplines, *E. coli* (Poolman & Wacker, 2016) and *S. aureus* (Zhao et al., 2016) are hazardous foodborne pathogens, that have caused many public poisonings and should be diagnosed and controlled (Wei et al., 2018). *E. coli* is usually known as a pathogenic indicator organism since it is present in humans and, in particular, dairy animal ingesta, (Morgan et al., 2008), which are shed daily to the environment. Its presence in milk indicates faecal contamination (Elmonir et al., 2018) while *S. aureus* impurity of milk could be related to infected dairy animals, acting as carriers of the pathogen (Kadariya et al., 2014) or livestock staff, chiefly those with poor sanitation, especially for primitive livestock husbandry practices (Abebe et al., 2016). In subclinical mastitis, in lactating dairy animals, both pathogens are shed into the milk (Bihon et al., 2019) and the health of the consumers of this milk can be affected (Elmonir et al., 2018).

Proper packaging materials should have high blockade attributes to decrease the frequency of this inappropriate process and increase the declared shelf life (Karaman et al., 2015). Investigations have shown some successfulness in increasing the shelf life of butter. It can be packed in polypropylene or LDPE, in aluminium foils (Karaman et al., 2015), with a nanocomposite (NC), such as cellulose-based papers coated in chitosan-Ag/TiO2 (Apjok et al., 2019) or in form of active packaging layer integrating anti-pathogenic nanocomposite (NC) included herbal compounds (Karaman et al., 2015) or omega-3 (São José et al., 2019). Butter is also gamma irradiated which improve the quality and increase the shelf life of the product (Rady & Badr, 2003). The first compounds applied as active packaging were organic acids, and non-degradable polymers. Currently, nanoparticles (NPs) have been extensively used with more benefits than other known combinations such as organic acids or enzymes due to high resistance to heat or severe chemical conditions (Simbine et al., 2019). Nanoparticles are suggested to have anti-microbial activity due to their prevention of aerobic respiration and damaging the DNA of pathogens (Shi et al., 2018), reactive oxidative stress on the cell membrane of bacteria, and binding to the cell membrane convinced by AgNPs (Yan et al., 2018).

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Based on the aforementioned references, the objective of our study was to increase the shelf life of both pasteurised and traditional butters using Ag-LDPE of different concentrations as films to cover the butter stored at a refrigeration temperature and analyze the bio-chemical parameters of the butters.

2 Materials and methods

2.1 Preparation of silver nanoparticles (AgNPs)

Silver nanoparticles were synthesized through the chemical reduction following the method of Vazquez-Muñoz et al. (2019). The 0.849 g of $AgNO_3$ (Sigma-Aldrich Sydney, Australia) was dissolved in 50 mL of distilled water, and the solution was boiled. Trisodium citrate (1 gr) was then dissolved in 100 mL of distilled water. The 5 mL of the later solution were trickled to 100 mL of boiling $AgNO_3$ solution and heated at 90 °C for 2 hours. It was then cooled at room temperature to give a reddish solution.

2.2 Preparation of silver nanocomposite (NC) film

Low Density Polyethylene (LDPE), with melt index of 25 g/10 min (190 °C/2.16 kg) was prepared (Sigma-Aldrich 9002-88-4, Germany). In order to stabilize the dispersity of particles into the LDPE matrix, nanosilver samples at concentrations of 0%, 2.5%, 7.5%, 12.5% and 17.5% were dissolved in polyethylene glycol monostearate (PGE) (Sigma-Aldrich, Germany) using a sonication bath for 10 min. Through the melt mixing process, the produced PGE-nanoparticles were exposed to the LDPE matrix at 130 °C following the method of Jokar et al. (2012) with minor modification. A hot plate with a pressure of 45 bar was used at 135 °C for 12 min to obtain square samples, followed by 12 min of cooling time according to the method of Jokar et al. (2012) with minor modifications. The Ag-NC packaging coatings were first cut to 5×5 cm and sterilised by a UV lamp for 20 min before use.

2.3 Scanning Electron Microscopy (SEM) test

Morphology and size of Ag-NP was analyzed using scanning electron microscope (SEM, TESCAN, MIRA3, Brno, Czech Republic). The images produced in the composite field, in which a suspension was primarily prepared in an acetonitrile solvent in a lab Falcon and 3 mL of the resulting solution then placed on the adhesive base to evaporate the solvent. Subsequently, it was transferred to the Spotter coater and coated with gold exposed to argon gas for 10 m. Ultimately, the final sample was placed into the chamber of the SEM and bombardment with fast electrons was done on the surface of the sample and images were recorded (Martins et al., 2012).

2.4 Preparation of bacteria

In the current study, the numeration of total viable bacteria, *Escherichia coli* (ATCC 25922), coagulase-positive (CP) *Staphylococcus aureus* (ATCC 9144), and psychrophilic bacteria was performed. The bacteria were prepared from the Iranian Research Organization for Science and Technology (IROST) and transferred to the laboratory of the Department of Food Science and Technology, IAU. The considered culture existed in plastic bead was added into a tube containing 5 mL of Nutrient Broth to revitalize the lyophilized bacteria by incubating at 37 °C for 24-48 h. (Shrivash et al., 2018). The working stock culture was kept on tryptone soy agar at a refrigeration temperature.

2.5 Preparation of butter samples

Pasteurized and traditional butters were purchased from a pasteurized dairy company and a dairy retailer, respectively. They were transported to the laboratory under refrigeration temperature and then aseptically packed (10 g packages weighted from all parts of the surface, centre, and bottom of the butters) with Ag-nanocomposites (Ag-NC) at 0%, 2.5%, 7.5%, 12.5% and 17.5% in triplicates and stored at 4 °C for one month. The butter samples were inoculated with the bacterial strains before packing. Thus, each butter sample was inoculated with 1 mL of suspension of 0.5 McFarland (1.5×10^8) of each *S. aureus* and *E. coli* prepared from the working solution (Institute of Standard and Industrial Research of Iran, 2012b). After one month, the samples were kept under sterile conditions for 20 minutes at room temperature to soften slightly. The butter content of each package was poured into an Erlenmeyer and placed in a water bath to be melted at 35 °C. An amount (90 mL) of 40 °C-sterile peptone solution was added 10 g of melted butter, stirred and some samples were then removed from the aqueous phase for bacterial culture (Sarab et al., 2019). The 6-fold serial dilution was then prepared.

2.6 Faecal coliform diagnosis and confirmation tests

In order to detect coliforms (Bereda et al., 2018; Feng et al., 2002), 0.1 mL of the suspension (named final suspension) was removed from each of the last three diluted tubes (10^{-4}, 10^{-5}, 10^{-6}) and poured into a sterile plate pre-filled with 10 cc of Violet Red Bile Agar (VRBA) medium, and two-layered culture was carried out through the pour plate method. The plates were then incubated at 37 °C for 48 h and numerated for CFU based on the dilutions and CFU counted in the plate. A few purple colonies with bile halo grown in the VRBA medium were removed and aseptically transferred to sterile brilliant green bile (BGB) and lactose broth medium (Endol test). They were incubated at 37 °C for 48 h. After incubation, a red ring at the ambient surface appeared after adding a few drops of Kovacs' reagent to tubes containing the latter medium. An opaque colour then formed in BGB environment, along with the presence of gas in the Durham tubes, thus confirming the presence of coliforms in the samples. A loop of bacteria grown in the last positive tubes were removed, linear culture was performed on the Eosin Methylene-Blue Lactose Sucrose Agar (EMB, Sigma Aldrich, Germany) and incubated at 44.5 °C for 24 h to confirm fecal coliform.

2.7 Coagulase Staphylococcus test

The 0.1 mL of the final suspension was removed, inoculated the surface of sterile plate pre-filled with 10 cc Baird Parker agar (BPA Sigma Aldrich, Germany) and incubated at 37 °C for 24 h. The plates were counted with a glossy black colony with a thin white edge and clear halo (Sarab et al., 2019).

2.8 Psychrophilic bacteria test

The 0.1 mL of the final suspension was removed and added on to the surface of each sterile plate pre-filled with 10 cc Plate

Count Agar (PCA, Sigma Aldrich, Germany). They were then kept at refrigeration temperature (6.5 °C) for 10 days. White spindle-shaped colonies were counted (Sarab et al., 2019).

Bacterial Total Count (TC)

The 0.1 mL of the final dilution was inoculated on each plate containing 10 mL of plate count agar (Sigma Aldrich, Germany). After the agar was firmed, the plates were inoculated at 37 °C for 36-48 h (Bereda et al., 2018).

2.9 Moisture content

A specific weight of butter was heated at 102 °C ± 2 °C. The dry matter was weighed as moisture content. The volume of percent moisture was calculate using Equation 1:

$$Wm = \frac{(ml - m2)}{(ml - m0)} \times 100 \quad (1)$$

where *Wm* is the volume of moisture content (g 100 g^{-1} butter, as %), m_0 is the weight (g) of the bare beaker, m_1 is the weight (g) of beaker and butter contained therein before heating and m_2 is the weight (g) of beaker and butter contained therein after heating (Evers et al., 2003).

2.10 Solid-non-fat (SNF) content

The volume of percent SNF was calculated using Equation 2:

$$Ws = \frac{(ml - m0)}{(m2 - m0)} \times 100 \quad (2)$$

where *Ws* is the volume of SNF content (g 100 g^{-1} butter, as %), m_0 is the weight (g) of the bare beaker, m_1 is the weight (g) of the bare beaker along with dried SNF and m_2 is the weight (g) of the butter sample along with the beaker (Evers et al., 2003).

2.11 Content of fat-by-difference

The volume of percent Fat-by-difference was calculated using Equation 3:

$$Wf = 100 - (Wm - Ws) \quad (3)$$

where wf; wm and ws are the volume of fat (g 100 g^{-1} butter, as %), volume of moisture (g 100 g^{-1} butter, as %) and the the volume of SNF (g 100 g^{-1} butter, as %) respectively (Evers et al., 2003).

2.12 Fatty acid composition

Fatty acid (FA) content was measured using Gas Chromatography (GC). The 500 µL hexane and 200 µL of 2 N KOH in methanol were macerated into the control and treatment tubes each containing 50 µL of the butter to derivate it to fatty acid methyl esters (FAME). The combination was stirred up to 5 min and the upper organic phase that contained FAME was three times extracted for evaluation using a gas chromatography equipped with a flame ionization detector (Agilent 6890 N Hewlett-Packard Co., Avondale, PA, USA) (FID) and a capillary column with dimensions of 100 m × 0.25 mm × 0.2 µm. Respectively, air flow and hydrogen levels were set to 300 and 30 mL min^{-1}. Results were ultimately presented as w⁄w (%) total FA (Bali et al., 2017).

2.13 Peroxide test

Peroxide value of the butter samples was measured following the method presented by (AOAC standard methods CD-8b-90) (21). The 30 mL combination of glacial acetic acid and chloroform (3:1 v/v) solution was macerated into the Erlenmeyer flask containing 5 g of butter samples and slightly stirred, followed by adding 0.5 mL of the saturated potassium iodide. The solution was added with 30 mL of deionized water after it was left in darkness for a minute. The 0.01 N sodium thiosulphate solution was added to the combination to titrate until the yellow color disappeared. The next titration was carried out by adding 0.5 mL of the starch adhesive reagent (1% w/v) to the flasks, and constant shaking was continued until the blue color disappeared. The peroxide value (mEq/kg) was calculated according to the following Equation 4:

$$p = \frac{N(S - B)}{W} \times 1000 \quad (4)$$

where *p* is the peroxide number of butter sample (meq/kg butter), N is normality of the thiosulphate solution, S is the volume of sodium thiosulphate used by fat of samples (mL), B is the volume of sodium thiosulphate used by blank, and W is the sample was used (g).

2.14 Melting point test

Melting point of the butter samples was measured according to (AOCS method Cc1-25) (24). Capillary tubes were filled with a 1 cm high column of melted butter. The capillary tubes were then placed in an ice-filled beaker for a few seconds until they were being chilled in a cold bath to solidify the butter for 5 min. The tubes were then contacted to a thermometer, and placed in a beaker prefilled with distilled 15 °C-water. The bath temperature was set to 37 °C, and heat was applied to increase the temperature at a rate of 3-4 °C/min. As soon as the fat column rose in one of the two capillaries, the temperature was reported as melting point.

2.15 Iodine index measurement

The 10 cc of chloroform and 25 cc of Hanus reagent were macerated to 0.25 g of the butter sample (Issa et al., 2017). The mixture was left in the dark room for 30 minutes. Potassium iodide 15% was added and the solution was then titrated in the presence of starch adhesive with 0.1 normal thiosulfate (titration continued until white color appeared).

2.16 Acidity measurement (oleic acid base)

The 2 g of each butter sample was mixed with 30 cc of alcohol using magnetic stirrer and a few drops of phenolphthalein reagent were trickled to titrate with a 0.1 normal NaOH until a light pink color appeared (1).

2.17 Measurement of saponification index

The 2 g of each butter sample was transferred to Erlenmeyer flask containing 50 cc of KOH, placed in water bath using a reflex set for 30 min (Al-Bachir & Othman, 2019). A few drops of phenolphthalein reagent were trickled to titrate with a 0.5 normal HCl until a yellow color appeared.

2.18 Statistical analyses

A mixed model-ANOVA Repeated Measurement within the General Linear Model (GLM) procedure of SPSS version 18 (SPSS Inc., IL, USA) was used to control which interaction could be significant for dependent variables in the artificially bacterial contaminated butters. When *p*- values were less than 0.05, the difference between the individual estimated marginal mean was significant using an ANOVA specific model followed post-hoc Tukey's HSD test ($p < 0.05$). A Univariate analysis was followed while the former model would not be significant ($p > 0.05$).

3 Results and discussion

3.1 SEM Test

Particle-size distribution with 20 kV and magnification of 200 kx are shown in Figure 1. In the image examined with SEM, agglomeration was extremely low, and the homogeneity of the coating surface was evident. The Ag-NPs size of nanocomposite coatings was ranged from 15.97 to 25.86, and the average particle size was 20.63 nm (Figure 1). To date, there are few studies have investigated the association between nano/LDPE composites and shelf life of the butter. The size of nanoparticles of the packaging is a crucial factor for the production of nanocomposites. The average size of nanoparticles produced throughout the chemical reduction method (Figure 1) was 20.63 nm, which is similar to those of other researchers have evidenced on NPs production size of 23.8 nm (Goharshadi & Azizi-Toupkanloo, 2013), 21 nm (Van Dong et al., 2012), and the size of nanoparticles between 10 and 30 nanometers (Khan et al., 2011). Small nanoparticles containing 10-30 nm in diameter exhibited more antibacterial properties than larger nanoparticles (Jeong et al., 2014).

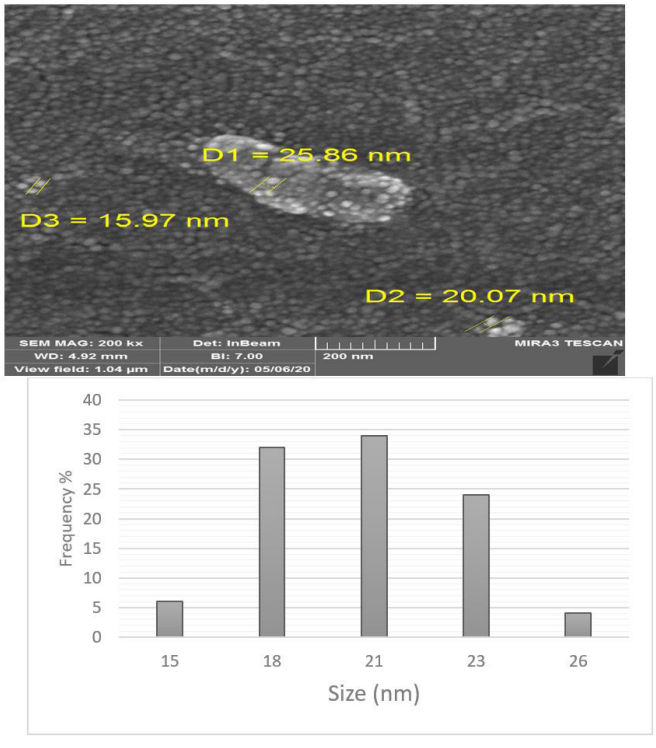

Figure 1. The scanning electron microscope (SEM) of 17.5% (Above × 200 kx) and frequency distribution (Below) showing the size of Ag-NC film. D: Diameter, D1: The largest diameter; D2: Medium Diameter; D3: The Smallest Diameter.

3.2 Microbial tests

Table 1 shows the microbial status obtained from the Pa and Tr butters wrapping in different Ag-LDPE nanocomposite (Ag-NC) films through the storage time. These data implied on the reduction of total bacteria count (TBC), *S. aureus, E. coli*, and psychrophilic bacteria with Ag-LDPE concentration-dependent manner so that the TBC of Pa butter was 14.66×10^4 CFU/g in the control group on the first day and gradually reached 7.33×10^4 CFU/g in the butter enfolded in 17.5% Ag-LDPE. It was remarkably decreased and reached "Not Determined" (ND) in the Pa butters enfolded with 12.5% and 17.5% Ag/LDPE films after 30 days of preservation. The TBC of Pa butters inoculated with 2%*Origanum acutidens* or 2% T*hymus haussknechtii* extracts reached 831.76×10^4 and 309.02×10^4 CFU/g after 30 days at 4 °C (Dagdemir et al., 2009) more remarkable than those gained in this study and another research conducted on the effect of gamma irradiation at a dose of 2.5 kGy exposing to Pa butter samples (Rady & Badr, 2003), which showed that the TBC reached 66×10^4 CFU/g at refrigerating condition. The discussion as mentioned earlier confirmed that nanosilver coating at 12.5% and 17.5% had good effectiveness in reducing bacterial load in Pa butter during 30 days of cold storage.

On the other hands, TBC was decreased (83.33 and 16.00×10^4 CFU/g) in Tr butter wrapped with 12.5% and 17.5% Ag/LDPE films after 30 days (Table 1) but significantly greater ($p < 0.05$) than the standard level (7.5×10^4 CFU/g). Unlike with TBCs of butter wrapped with 12.5% and 17.5% Ag-LDPE films, the values of other groups showed an increase from the first to thirtieth day in Tr butters. High TBC in Tr butter could be due to a high bacterial load of applied milk, lack of pasteurization and technology of heating, and the effect of both segregation and agitating procedures of butter production on the breakaway of bacterial mass result in increases their number (Gazu et al., 2018). Result of a research (Idoui et al., 2010) showed TBC of samples isolated from Tr cow butter ranged from 15×10^4 to 600×10^4 CFU/g, which was relatively in agreement with this result showed 191×10^4 CFU/g at the first day more sumptuous than the samples were taken from Tr Tunisian butters, which it gone 7.00×10^4 CFU/mL at the same time (Samet-Bali et al., 2009). TBC in Pa butter was reached 2.49×10^3 CFU/g after four weeks of cold storage while green tea extract was inoculated to butter (Thakaeng et al., 2020), showing less value compared to this study (Table 1).

The result of *E. coli* and *S. aureus* count (SC) was ND before the study for both Pa and Tr butters (Not given in the

Table 1. Estimates of bacterial count (×10⁴ cfu/g) isolated from butters (Mean ± SE) covered with different concentration of Ag-nano composite films during the storage (n=3).

Bacteria	D	But	Ag-nanocomposite%					SE	NS (Institute of Standard and Industrial Research of Iran, 2012b)
			0	2.5	7.5	12.5	17.5		
Total count ***	1	P	14.66^{aA}	11.66^{bA}	8.66^{cA}	8.33^{c}	7.33^{c}	3.43	$<7.5\times10^4$
		T	191.00^{aA}	180.00^{bA}	127.66^{cA}	111.00^{dA}	86.66^{eA}	3.43	
	30	P	229.66^{aB}	87.00^{bB}	35.00^{cB}	ND	ND	3.43	
		T	290.66^{aB}	260.66^{bB}	229.66^{cB}	83.33^{dB}	16.00^{eB}	3.43	
Staphylococcus C+ ***	1	P	19.33^{aA}	5.00^{bA}	5.00^{bA}	4.00^{bA}	ND	1.07	0
		T	9.00^{aA}	9.00^{aA}	1.33^{bA}	2.13^{bA}	2.66^{bA}	1.07	
	30	P	20.66^{aA}	12.33^{bB}	4.66^{cA}	4.66^{cA}	ND	1.07	
		T	270.66^{aB}	175.66^{bB}	35.57^{cB}	69.33^{dB}	54.00^{eB}	1.07	
E. coli ***	1	P	33.33^{aA}	31.00^{aA}	31.66^{aA}	6.33^{b}	4.75^{b}	0.64	0
		T	285.00^{aA}	280.00^{aA}	290.00^{aA}	290.00^{aA}	22.50^{bA}	0.64	
	30	P	33.00^{aA}	31.00^{aA}	6.33^{bB}	ND	ND	0.64	
		T	38.33^{aB}	32.33^{bB}	32.00^{bB}	2.00^{cB}	1.33^{cB}	0.64	
Psychrophilic b. ***	1	P	163.33^{A}	ND	ND	ND	ND	4.76	10^4
		T	203.33^{A}	ND	ND	ND	ND	4.76	
	30	P	171.66^{B}	ND	ND	ND	ND	4.76	
		T	290.66^{aB}	266.00^{b}	260.33^{b}	110.33^{c}	79.00^{d}	4.76	

But: Butter, D: Days; C+: Coagulase-positive; P: Pasteurized; T: Traditional; ND: Not determined; NS: National standard; SE: standard error. Different small superscripts in each row indicate a significant difference ($P < 0.05$). Capital superscripts in each column (for comparing between the values of same butter type and day) indicate a significant difference at a p value of 0.05. For interaction of dependent variables *p < 0.05, **p < 0.01 and ***p < 0.001

Tables). Once inoculation of *S. aureus* to butter was done, the SC reached 19.33×10⁴ CFU/g in Pa butters (Table 1) of the control group on 1st day, gradually decreased ($p < 0.05$) in butters with different concentrations of Ag-LDPE and reached the least values (4.00 ×10⁴ CFU/g and ND), respectively in 12.5% and 17.5% Ag-LDPE films. Similarly, this value declined and reached 4.66 ×10⁴ CFU/g and ND isolated from samples of 12.5% and 17.5% Ag-NCAg-LDPE films after 30 days. The SC for Tr samples started from 9.00×10⁴ CFU/g in the control group and ended at 2.66 ×10⁴ CFU/g ($p < 0.05$) in 17.5% Ag-LDPE film samples. The SC was increased more than twenty-thirtyfold after a month of cold preservation in Tr butters wrapped with different concentrations of Ag/LDPE films. This result indicated that only the 17.5% Ag/LDPE film could eliminate *S. aureus* from Pa butter samples. The results (Table 1) showed less capability of Ag-LDPE on reducing *S. aureus* value in Tr butter in comparison with Pa butter. Thus, this value for Tr butters decreased from 270.66 ×10⁴ CFU/g on the1st day to 54.00 ×10⁴ CFU/g after 30 days of the cold preservation. The value of *S. aureus* isolated from Tr butter was about 1×10⁴-10⁶ CFU/g (Mehdizadeh et al., 2019; Rahimi, 2013) which could be diminished by 2.8 log CFU/g after inoculation with 0.5% WHE (walnut kernel septum membranes hydroalcoholic extract) reached 1.3×10⁴ CFU/g after 45 days (Mehdizadeh et al., 2019). Contrarily, Sarab et al. (2019) showed coagulase-positive *S. aureus* isolated from Tr cow butters was reached 0.12 CFU/g without initial bacterial inoculation. Accordingly, 17.5% Ag-LDPE film could eliminate *S. aureus* in Pa butter at the end of storage vs. Tr samples of which the Ag-LDPE films could not effectively eliminate *S. aureus*. Our result demonstrated that the value of *S. aureus* was gradually diminished after inoculation, from 20.66×10⁴ ± 1.07 CFU/g (0% Ag-LDPE) to ND (17.5% Ag-LDPE) in Pa butter on day 30 indicating the increase effectiveness of Ag-LDPE film on reducing *S. aureus* in a dose-dependent manner.

The value of *E. coli* in Pa butter was 33.33×10⁴ CFU/g in the control group on the first day with no significant difference ($p > 0.05$) compared to that of the same group on day 30. This value for Pa butters covered with 7.5%, 12.5% and 17.5% Ag-LDPE were decreased on day 30 (6.33 ×10⁴ CFU/g, ND and ND, respectively) while the values for Tr butters were 32.00, 2.00, and 1.00 ×10⁴ CFU/g, respectively. on 30th day (38.33 ×10⁴ CFU/g), which later showed no significant difference ($p > 0.5$) with those of 2.5% Ag-LDPE (32.33 ×10⁴ CFU/g) and 7.5% Ag-LDPE (32.00 ×10⁴ CFU/g) and significantly decreased ($p < 0.05$) at 12.5% and 17.5% Ag-LDPE butters (2.00 ×10⁴ CFU/g and 1.33 ×10⁴ CFU/g, respectively) but did not reach ND. Notwithstanding the value of inoculation to the butters, the Pa butter covered with 12.5% and 17.5% Ag-LDPE films could eliminate *E. coli* from the butter. This result (Table 1) indicated that the efficiencies of Ag-LDPE films on diminishing *E. coli* from the Pa butters were more superior to that of the Tr ones. Active cellulose-based papers loaded in chitosan-Ag/TiO2 was suggested (Apjok et al., 2019) to be effective to reduce *E. coli* (2 CFU/g) from clarified butter relatively similar to this result showed not only 12.5% and 17.5% Ag-LDPE films to fully diminish *E. coli* in Pa butter after 30 days (ND) but also they decreased *E. coli* in Tr butters by 4.5 and 4.12 log CFU/g, respectively. A dose of 2.5 kGy gamma irradiation exposed to Pa butter samples could not inhibit the growth of coliforms, so that it was measured 120.0 CFU/g on the 1st day and reached 730.0 CFU/g on day 30 against 5 kGy gamma irradiation showed no coliforms could grow (ND) in the Pa butter (Rady & Badr, 2003) similar to the results of adding 0.5% WHE (Mehdizadeh et al., 2019) and 12.5%

and 17.5% Ag-LDPE films of this study (Table 1) showing *E. coli* were completely diminished in the Pa butters.

The result of psychrophilic bacteria was listed in Table 1. It showed that the bacteria would be sensitive while the Pa butter wrapped in the different concentrations of Ag-LDPE films even after 30 days so that its value showed no bacteria (ND) in the Pa butter samples enfolded different concentrations of Ag/LDPE film. The psychrophilic bacteria showed proper growth in the Tr butter samples wrapped even with 17.5% Ag-LDPE films (79.00×10^4 CFU/g). This result (Table 1) exhibited that Ag/LDPE film at different concentrations was effectively eliminated psychrophilic bacteria in Pa butters preserved in refrigerated temperature after 30 days while it showed no efficiency against Tr bacteria in the same condition. On the other hand, the 0.5%WHE could not effectively remove psychrophilic bacteria from the Pa butter so that its value reached 5.07 log CFU/g after 45 days of the storage (Mehdizadeh et al., 2019). Dairy product shelf life was intensified by low oxygen atmospheres due to depletion in the spoilage microorganisms including aerobic Gram-negative psychrotrophic bacteria, particularly *Pseudomonas* spp (Singh et al., 2012). Accordingly, the most antibacterial effect of an adequate dose of Ag^+ is notably observed in Gram-negative bacteria demonstrating that Ag^+ directly interacts with and disrupts Fe-S clusters in the outer membrane (Morones-Ramirez et al., 2013), results in low values of *E. coli* (Table 1).

3.3 Physicochemical tests

Table 2 displays the physicochemical properties of the commercial preparation of Tr and Pa butters. Out of the independent variables mentioned in Table 2, the interaction of time and Ag-LDPE film showed the maximum effectiveness on moisture, SNF, and Fat ($p < 0.05$; $\eta p2 = 0.30$, $\eta p2 = 0.985$, $\eta p2 = 0.985$; respectively). Initially, the moisture value (%) of Tr Butter of the control group as well as the treatments, was significantly ($p < 0.05$) more generous than those of Pa one in the same group. On the other hand, the data (Table 2) indicated that moisture value (%) of the butters (Pa or Tr) with different Ag-LDPE films on day 30 had no significant difference ($p > 0.05$) compared to that of the initial day. The moisture of Pa and Tr butters covered with 17.5% Ag-LDPE was 14.22% and 15.69%, which had no significant difference ($p > 0.05$) with those of other concentrations Ag-LDPE films. The minimum and maximum moisture values were 14.78% and 14.50%, respectively for control and 17.5% Ag-LDPE film on day 30.

Similar to our results, the moisture value of butter approximately was 15.00% (Mehdizadeh et al., 2019), which was less than the standard (Institute of Standard and Industrial Research of Iran, 2012a). In addition to fats, butter encompasses low portions of proteins and moisture making it a proper environment for microbial growth (Rady & Badr, 2003). The LDPE is a polymer that is widely used in food packaging. The advantages of this polymer are permeability to oxygen and very low impermeability to water and moisture (Dirim et al., 2004), which could be an apparent response to the inalterability of moisture in Pa and Tr butters after 30d. Regardless of butter type, the water content of the butters showed no significant difference ($p > 0.05$) within the treatments ranging from 14.94% to 14.98% on the beginning of the study and 14.50 to 14.78 after 30 days of the study. Development of bacterial spoilage is a rancidity process of butter complications, which can be produced through hydrolysis. Many psychrophilic bacteria, such as *S. aureus,* have been involved in lipolysis at low temperature of preserved butter (Rady & Badr, 2003). Butter with 80-82% lipid element is a palatable lipid percentage throughout the world (Mofid et al., 2018), which is in agreement with this result (Table 2) showed the fat content is approximately ranging 82.81-84.35%.

The SNF value (%) of the butters had no any significant changes ($p > 0.05$) after 30 days of the cold storage (Table 2) so that the SNF value of Pa and Tr butters on the 1st day was ranging 1.22-1.25% and 1.53-1.58%, respectively with no significant difference ($p > 0.05$) compared to those of 30th day. These results showed that Tr butter had insignificantly ($p > 0.05$) more SNF than Pa one. The SNF (%) of Tr samples was greater than that of Pa butter (Table 2). It could be due to the process of traditional butter production, in which butter extraction is prepared from the yoghurt results in infiltration of phosphorus,

Table 2. Gross composition (w/w (%) of butters (Mean ± SE) at different concentration of Ag-nano composite films during the storage.

Bacteria	D	But	Ag-nanocomposite%					SE	NS
			0	2.5	7.5	12.5	17.5		
Moisture (%)	1	P	14.23^{aA}	14.22^{aA}	14.24^{aA}	14.23^{aA}	14.22^{aA}	0.02	<16%
		T	15.71^{aB}	15.66^{aB}	15.70^{aB}	15.73^{aB}	15.69^{aB}	0.02	
	30	P	14.05^{aA}	13.98^{aA}	13.88^{aA}	13.80^{aA}	13.73^{aA}	0.02	
		T	15.53^{aB}	15.45^{aB}	15.38^{aB}	15.32^{aB}	15.26^{aB}	0.02	
SNF (%)	1	P	1.25^{aA}	1.23^{aA}	1.25^{aA}	1.22^{aA}	1.22^{aA}	0.01	<2%
		T	1.57^{aA}	1.58^{aA}	1.53^{aA}	1.58^{aA}	1.58^{aA}	0.01	
	30	P	1.29^{aA}	1.25^{aA}	1.24^{aA}	1.24^{aA}	1.28^{aA}	0.01	
		T	1.66^{aA}	1.66^{aA}	1.66^{aA}	1.66^{aA}	1.65^{aA}	0.01	
Fat (%)	1	P	84.39^{aA}	84.29^{aA}	84.40^{aA}	84.35^{aA}	84.33^{aA}	0.01	>82%
		T	82.87^{aB}	82.76^{aB}	82.79^{aB}	82.80^{aB}	82.80^{aB}	0.01	
	30	P	84.37^{aA}	84.29^{aA}	84.30^{aA}	84.33^{aA}	84.31^{aA}	0.01	
		T	84.37^{aA}	84.29^{aA}	84.30^{aA}	84.33^{aA}	84.32^{aA}	0.01	

But: Butter, D: Days; SE: standard error; NS: National standard; SNF: solid-non-fat; P: Pasteurized; T: Traditional; Different small superscripts in each row indicate a significant difference at a p value of 0.05. Capital superscripts in each column (for comparing between the values of same butter type) indicate a significant difference at a p value of 0.05. For interaction of dependent variables *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

calcium, and protein to ultimate butter (Mehdizadeh et al., 2019). According to Table 2, the SNF values for both butters were less than the standard (Institute of Standard and Industrial Research of Iran, 2012a). According to EU Council Regulation No. 2991/94, butters should enclose at least 80% fat, less than 16% water and 2% SNF (European Union, 1994). The value of SNF of traditional cow butter was 1.90% relatively more than those of the Tr samples of this study (1.57).

On the first day, the fat content (%) of the Pa and Tr butters (84.39% and 82.87%, respectively) of the control showed no significant difference ($p > 0.05$) compared to corresponding butters wrapped with different concentrations of Ag/LDPE films. Its value of Pa butters showed no significant difference ($p > 0.05$) in different concentrations of Ag/LDPE after 30 days of cold storage. Still, it was increased (84.37%) in Tr butters of control with no significant difference ($p > 0.05$) in comparison to the treatments at the same time (Table 2). Fat and SNF values of Irish summer butter were 82.15% and 1.96%, respectively (Cronin et al., 2007).

Fatty acid compositions of the butter samples are presented in Table 3. According to ANOVA repeated measure test, the impact of different concentrations of Ag/LDPE films had no significant effect ($p > 0.05$) on the FFA content of both butters. However, lipid content showed that levels of the short-chain FAs (C4:0–C12:1) were lower in Pa butter (13.65%, w/w) than that of Tr butter (16.58%, w/w). Accordingly, saturated FAs were calculated 70.15% (w/w) and 72.79% (w/w), respectively for Pa and Tr butters in the initial day. They reached 68.65% (w/w) and 73.06% (w/w), respectively after 30 days of cold storage. Monounsaturated FAs (MUFA) of Pa butter (26.46%, w/w) was remarkably more significant than that of Tr butter, approximately was 22.31%, w/w. The higher proportion of FA was observed for palmitic acid, C:16 (33.94%, 34.82% w/w,) followed by oleic acid, C:18.1 (23.53%, 18.44%, w/w) and stearic acid, C:18 (11.50%, 12.27%, w/w), respectively in Pa and Tr (Table 3). Regardless the concentration of Ag-LDPE film, the values of FA content of both Tr and Pa butters did not change during the storage exception for Linoleic acid, which is an essential polyunsaturated omega-6 FA for humans. The value of C18:2 FA of both Pa and Tr butters on the 30th day (4.90 ± 0.02, 4.63 ± 0.02, respectively) showed an increase compared with the initial day (3.40 ± 0.02, 4.90 ± 0.02, respectively). Dislike, FFA content increased within the study days sampled from butters affected by extracts of sage, rosemary, and oregano (Ayar et al., 2001). In our study, Palmitic acid content of Pa and Tr butters respectively, were 33.9% and 34.8% greater than that of butter of cows fed on flaxseed (25.8%) with no changes that occurred after one month of storage (Silva-Kazama et al., 2010). On the other hand, the value of stearic acid, which it is a waxy saturated fatty acid, was decreased after 45 days from 16.88% to 16.34% (Silva-Kazama et al., 2010) in comparison with this study represented 11.50% and 12.27% after 30 days with no remarkable changes against the initial day.

The results obtained from the preliminary analysis of physicochemical criteria can be observed in Table 4. Initially, PV of the Pa butter (0.56 meq/kg) was significantly greater ($p < 0.05$) than those of Tr butter samples (0.30 meq/kg). These values showed no changes ($p > 0.05$) by increasing the percent of

Table 3. Fatty acid compositions (w/w (%)) of butters (Mean ± SE) at different concentrations of Ag-nano composite films during the storage.

FFA	Day	Butter	SE	NS
C4	NA	P 4.48^{a}	0.05	1-5
		T 5.58^{b}	0.05	
C6	NA	P 2.47^{a}	0.05	0.8-3.6
		T 2.61^{a}	0.05	
C8	NA	P 1.45^{a}	0.03	0.5-1.8
		T 1.78^{a}	0.03	
C10	NA	P 2.11^{a}	0.03	1.7-3.9
		T 2.80^{a}	0.03	
C12	NA	P 3.14^{a}	0.05	2.2-4.5
		T 3.81^{a}	0.05	
C14	NA	P 8.42^{a}	0.03	5.4-14
		T 9.51^{b}	0.03	
C14:1	NA	P 1.69^{a}	0.04	0.5-1.8
		T 1.80^{a}	0.04	
C16	NA	P33.94^{a}	0.07	22-41
		T34.82^{a}	0.07	
C16:1	NA	P 1.23^{a}	0.02	0.7-6
		T 2.07^{a}	0.02	
C18	1	P11.50^{aA}	0.02	6-15
		T11.50^{aA}	0.03	
	30	P11.50^{aA}	0.02	
		T12.27^{aB}	0.03	
C18:1		P 23.53^{a}	0.03	18-38
		T 18.44^{b}	0.03	
C18:2	1	P 3.40^{aA}	0.02	0.7-5.5
		T 4.90^{bA}	0.03	
	30	P 4.90^{aB}	0.02	
		T 4.63^{aB}	0.03	
C20	NA	P 0.04^{a}	0.02	0.05-1
		T 0.80^{b}	0.03	

NS: National standard; P: Pasteurized; T: Traditional; N.A.: Not affected associated with dependent variable (s); SE: standard error. Different small superscripts in a raw for C16 (for other FFA: Free Fatty Acid compares between the values at same day in a column) indicate a significant difference at a p value of 0.05. Capital superscripts in each column (for comparing between the values of same butter type) indicate a significant difference at a p value of 0.05. For interaction of dependent variables *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Ag/LDPE film. It was lower than the national standard for butter, which should be less than 1.0 meq/kg (Institute of Standard and Industrial Research of Iran, 2012a). Accordingly, the trend of the PV showed a slight fall from 0.50 and 0.28 meq/kg, respectively for Pa and Tr butters on the 30th day in the control group to 0.31 and 0.24 meq/kg, respectively in the 17.5% Ag-LDPE film at the end of the storage with no significant difference ($p > 0.05$) with other treatments. Off-flavors coincide with an increase of oxidation levels, which indicates higher PV (Krause et al., 2008). This value is considered to be one of the most indicators to show the steps of oxidative rancidity of lipids. Other researchers (3) confirmed that tomato processing by-product extract at 800 mg/kg showed more efficiency (1.31 meq/kg) to decrease PV in Tr Tunisian butter than 200 mg/kg butylated hydroxytoluene, BHT (4.38 meq/kg) at cold storage. The uses of bagasse adsorbent reduced PV up to 26.67% in frying oil (Kaltsum et al., 2016). Green tea extract could decrease PV of Pa butter reached 0.59 meq/kg after 28d

Table 4. Estimates of physicochemical properties of butters (Mean ± SE) covered with different concentration of Ag-nano composite films during the storage.

Property	D	B	Ag-nanocomposite%					SE	NS
			0	2.5	7.5	12.5	17.5		
Peroxide (meq/kg)	1	P	0.56^{aA}	0.58^{aA}	0.57^{aA}	0.58^{aA}	0.59^{aA}	0.66	<1
		T	0.30^{aB}	0.28^{aB}	0.29^{aB}	0.26^{aB}	0.24^{aB}	0.66	
	30	P	0.50^{aA}	0.49^{aA}	0.41^{aA}	0.31^{bB}	0.31^{bB}	0.66	
		T	0.28^{aB}	0.26^{aB}	0.28^{aB}	0.24^{aB}	0.24^{aB}	0.66	
Melting point (°C)	1	P	30.67^{aA}	30.66^{a}	30.33^{aA}	29.00^{aA}	30.00^{aA}	0.66	28-34
		T	32.00^{aAB}	31.66^{a}	32.67^{aB}	33.00^{aB}	33.00^{aB}	0.66	
	30	P	30.00^{aA}	30.00^{a}	30.00^{aA}	27.00^{bA}	28.00^{bA}	0.66	
		T	33.00^{aB}	32.00^{a}	32.66^{aB}	32.66^{aB}	32.00^{aB}	0.66	
Iodine (I_2 g/100g)	1	P	31.29^{a}	31.85^{a}	32.16^{aA}	30.95^{a}	30.32^{aA}	1.51	26-40
		T	29.56^{a}	29.49^{a}	29.53^{aA}	28.91^{a}	28.92^{aA}	1.51	
	30	P	30.72^{a}	31.34^{a}	31.00^{aA}	31.12^{a}	30.46^{aA}	1.51	
		T	28.52^{a}	28.04^{a}	27.83^{aB}	28.62^{a}	25.26^{bB}	1.51	
Acid Oleic base (%)	1	P	0.19^{a}	0.18^{a}	0.18^{a}	0.19^{a}	0.18^{a}	0.01	<0.3
		T	0.14^{a}	0.14^{a}	0.14^{a}	0.14^{a}	0.14^{a}	0.01	
	30	P	0.18^{a}	0.18^{a}	0.18^{a}	0.18^{a}	0.17^{a}	0.01	
		T	0.14^{a}	0.14^{a}	0.14^{a}	0.14^{a}	0.13^{a}	0.01	
Saponification (mg KOH/g)	1	P	236.61^{aA}	234.70^{bA}	234.44^{bA}	234.69^{bA}	234.55^{bA}	1.29	225-235
		T	234.55^{aA}	234.15^{aA}	233.06^{aA}	234.32^{aA}	234.22^{aA}	1.29	
	30	P	236.25^{aA}	231.45^{bA}	230.81^{bA}	229.65^{bA}	228.26^{bA}	1.29	
		T	233.47^{aA}	234.07^{aA}	232.28^{aA}	233.60^{aA}	233.88^{aA}	1.29	

B: Butter, D: Days SE: standard error; NS: National Standard; P: Pasteurized; T: Traditional. Different small superscripts in each row indicate a significant difference at a p value of 0.05. Capital superscripts in each column (for comparing between the values of same butter type) indicate a significant difference at a p value of 0.05. For interaction of dependent variables *p < 0.05, **p < 0.01 and ***p < 0.001.

at 4 °C (Thakaeng et al., 2020). Surprisingly, the value of PV of Pa butter was decreased by half and reached 0.31 meq/kg in groups of 12.5% and 17.5% Ag-LDPE films, respectively, showing a sensible decrease in oxidation level compared to other groups and Tr butter samples (Table 4). Similarly, the PV of the butter remained relatively stable, with increasing storage time up to one month in butter treated with WHE 0.5% at refrigerated temperature (Mehdizadeh et al., 2019).

In Table 4, there is an apparent decrease ($p < 0.05$) of melting point in Pa butter after 30d while the butters were wrapping with 12.5 and 17.5% Ag/LDPE films (27.0 and 28.0 °C, respectively) in which the melting point were 29.0 and 30.0 °C, respectively at the beginning of the study. There was no significant difference ($p > 0.05$) between the melting points of Tr butters on days 1 and 30. The butter stability can be improved by a higher melting point (34 °C) fractions from milk fat against low melting points butters (Schäffer et al., 2001). Analysis of the fat content and FFA profile of the Tr butter showed a greater melting point, and firmer in consistency that made it to be melted at higher points similar to yak butter (Neupaney et al., 2003), illustrating the higher melting point (41 °C) could be due to more significant fractions (58.35%) of palmitic acid and stearic acid, which relatively were dissimilar compared to this study showed its fraction was 45.44% and 47.09%, respectively for Pa and Tr butters (Table 4). The melting point of all butter samples dropped from 34.2 to 29.5 °C when up to 25% *Salvia hispanica* (chia) oil was gradually mixed with butter oil. Chia oil has a significant proportion of Omega-3 and 6 Fatty Acids (Rahman et al., 2015).

The iodine value of analyzed for both butters was given in Table 4. The iodine value of the Pa and Tr butter in control (31.29 and 29.56, respectively I_2 g/100 g) showed no significant difference ($p > 0.05$) compared to other groups (Table 4). Initially, the Pa butters were insignificantly ($p > 0.05$) shown greater iodine value than Ta butter in the same group. Except for Tr butters wrapped with 7.5 and 17.5% Ag/LDPE, which their iodine values respectively were decreased to 27.83 and 25.26 I_2 g/100g after 30d, Iodine values were not changed after 30d either for Pa or Ta butters (Table 4). The greater the iodine value, the more unsaturated fatty acids or greater double-bond number are present in the butter oil results in a decrease of melting point (Naghshineh et al., 2010), which is in agreement with the Pa butters showing greater iodine values (30.32 vs 28.92, respectively for Pa and Tr butters wrapped with 17.5%Ag/LDPE films) with lower melting point (30 °C). The iodine index of whey butter was higher with a lower melting point (31.2 °C) than regular butter (34.5 °C), which was due to the more significant proportion of unsaturated FA (Nadeem et al., 2015).

The acid value based on oleic acid (%) is presented in Table 4. Initially, the acid values of Pa and Tr butters were respectively 0.19% and 0.14% with no significant difference ($p > 0.05$) compared to those of butters wrapped with 2.5-17.5% Ag/LDPE films or in comparison to the values of the thirtieth day at the same groups showing no hydrolysis of FFAs of the butters. The lipolytic property of oil was defined as the volume of FFA (as oleic acid%), which was increased by hydrolysis of fat in the presence of water. The hydrolysis of fat was decreased over time due to moisture reduction (Koczoñ et al., 2008). In

a research (Tafreshi et al., 2015), the iodine value showed an increase of 2-fold in Pa butter wrapped with any concentration of rosemary extract-polymeric film after two months of cold storage dissimilar to the butter exposed to gamma irradiation (Rady & Badr, 2003) illustrated the acid value decreased by half reached 0.4% after one month against the result of this study (Table 4) showed no any changes in those of the acid value at the same time of storage. Since, the moisture value had no changes over the preservation, the stability of lipolytic activity could be due to the effect of Ag-LDPE films covered the butters (Table 2).

The main factors, which confirm fat quality, typical flavor, and odor in butters, are the saponification and acid values. The saponification value of Pa and Tr butters were 236.61 and 234.55 mg KOH/g ($p > 0.05$) in the control showed no significant difference ($p > 0.05$) compared to corresponding values of other groups. The values on the first day of cold storage insignificantly ($p > 0.05$) deceased on the thirtieth day, showing no remarkable difference in long-chain fatty acids (saturated and unsaturated) in both types of butters (Table 4). Long-chain FAs (saturated and unsaturated) are prone to be oxide and broken-down, which provides off-flavor and greater acid quantity showing the oxidation of triglycerides into FFA more generous in butter with high saponification values (Akhter et al., 2016).

4 Conclusions

It is concluded that LDPE film was produced in this study encompassed of the small size of nanosilver (20.63 nm) showed appropriate antimicrobial efficiency during cold storage up to 30d. Total bacteria, *S. aureus,* and *E. coli* were eliminated from the Pasteurized butters wrapped with 17.5% Ag/LDPE films. On the other hand, using psychrophilic bacteria can be eradicated with 2.5-17.5% Ag/LDPE films, which can be important for prohibition the lipolytic and oxidation of fatty acids in butters. Using the 12.5-17.5% Ag/LDPE films could significantly decrease peroxide value of pasteurized butter after 30d of cold storage showing a reduction of oxidative rancidity of lipids. The melting point of pasteurized butter insignificantly reduced by 2 degrees while the 12.5% and 17.5% Ag/LDPE film were used. Greater iodine value showed that the use of 17.5% Ag/LDPE film increased saturated fatty acid and made the stability of traditional butter more than pasteurized one. Ultimately, the use of 17.5% Ag/LDPE as a coating of butter can safely preserve pasteurized butter at least a month of cold storage, but it does not recommend to use Ag/LDPE film for protection of traditional butters.

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