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Original research

Combination effects of potassium lactate and sodium diacetate on the chemical and microbial attributes of hamburger during the frozen storage

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A B S T R A C T

This research study was accomplished for the extension of meat-based hamburgers during frozen storage. Control hamburgers were produced at three ground meat concentrations (30, 70, and 90%). Also, potassium lactate (PL) and sodium diacetate (SD) were added to the hamburger by 3% and 0.15%, respectively, to investigate their effects on moisture content, oxidative stability, and microbial growth. The results showed that the addition of PL and SD relatively maintained the moisture especially when meat ground was 70%. On this basis, after 150 days of storage, moisture was 59.70% for the hamburger with 70% compared with 58.56 obtained for the control. Higher oxidative stability was obtained for the hamburger with PL and SD and meat ground of 30% so that peroxide values were 2.49 and 1.97 meq O_2/kg, and 1.82 mg and 1.52 MDA/kg respectively for control and sample after 150 days of storage. The addition of PL and SD inhibited the growth of *salmonella*, limited the *E.coli*, molds, and yeasts growth.

Keywords: Hamburger; Potassium lactate; Sodium diacetate; Oxidative stability; Microbial growth

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1. Introduction

Nowadays, demand for ready-to-eat (RTE) products has been remarkably increased which is associated with machinery and industrial lives (Johnston et al., 2005; Muchaamba et al., 2021; Nikmaram et al., 2018). RTE products have a lower time to be prepared by the consumer and they can be ready for consumption after a few minutes of processing (for instance reheating) (Johnston et al., 2005). Their main preparation stages have been done in the respective food factories (Muchaamba et al., 2021; Nikmaram et al., 2018). Meat-based products such as hamburgers, salami, etc. are considered RTE products that can be prepared by reheating in a short time (Johnston et al., 2005; Muchaamba et al., 2021). One of the main challenges of these products is their storage preservation because they are perishable products (Muchaamba et al., 2021; Thomas et al., 2008). Indeed, some drawbacks such as oxidation of oil, microbial contamination, water or oil leakage, and drip loss encounter these products' consumption with limitation (Cheng et al., 2007). So, using recommended antimicrobial agents such as sodium chloride, sodium nitrite and nitrate somehow is inevitable (Kim et al., 2018; Lilic et al., 2015; Zhou et al., 2010). The technology termed “hurdle” has been developed in terms of using a simultaneous combination of two or more methods to control and inhibit the meat products' spoilage (Aymerich et al., 2005; Rahman, 2015; Thomas et al., 2008). For instance, the production of frozen hamburgers alongside using antimicrobial and antioxidant agents and/or using irradiation with natural plant extracts, or using potassium lactate with the treatment of high hydrostatic pressure and low storage temperature can increase the product storage with maintained nutritional quality (Aymerich et al., 2005; Johnston et al., 2005; Lee et al., 2005; Rahman, 2015; Zhou et al., 2010).

Sodium chloride (salt) has been also traditionally used for preserving meat and has positive effects on the taste, water holding capacity, and the reduction of water activity (Lilic et al., 2015; Muchaamba et al., 2021). According to the recent findings, a daily requirement in sodium for adults to maintain metabolic processes is below 1500 mg while daily intake of sodium is often over 5000 mg. The American Heart Association recommends for persons with hypertension that daily intake of salt should not be over 1500 mg,

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especially for persons with congestive heart disorders, it should not be more than 1000 mg (Aymerich et al., 2005; Lilic et al., 2015; Muchaamba et al., 2021).

Sodium nitrite has been extensively used in curing meat and meat products (Alahakoon et al., 2015; Gassara et al., 2016; Misra & Jo, 2017). It acts as a color fixative and inhibits the growth of bacteria, including *Clostridium botulinum*, which is the source of botulism toxin (Gassara et al., 2016). Nitrite is a relatively strong reducing agent that has antibacterial features. However, the preservation of foodstuffs can be attributed to a large degree to the high concentration of salts (including nitrate) that are employed during the curing process. One of the most important properties of nitrite is its ability in delaying the development of oxidative rancidity (Gassara et al., 2016). Although nitrate addition has a standard (FDA) regarding the addition concentration, some food processors use higher concentrations to control the spoilage or any microbial and chemical drawbacks, and subsequently, conversion of nitrite to nitrosamine and/or other aromatic substances during the heating process leads to metabolic and severe health disorders (Brender, 2020; Vlachou et al., 2020). It means strong inspection is being needed for inhibition of high-dose usage. However, it seems there are other good and efficient ways to control and prevent adverse reactions or microbial contamination. Considering these, there have been discovered other salts (without nitrate and sodium chloride) with healthy and more effective preservation properties. In this regard, potassium lactate and sodium diacetate have been reported as efficient salts to control and prevent chemical and microbial contamination (Aymerich et al., 2005).

Lactic acid has several ionic states with the dominant application of sodium and potassium lactate (Aymerich et al., 2008; COMA, 2008; Muchaamba et al., 2021; Zhou et al., 2010). Sodium lactate has been vastly used in the meat industry because of its ability to increase flavor, prolong shelf life, and improve the microbiological safety of products (Muchaamba et al., 2021; Zhou et al., 2010). The antimicrobial effects of lactates are due to their ability to lower water activity and the direct inhibitory effect of the lactate ion. Several researchers have successfully approved the extension effects of lactates regarding the shelf life (Zhou et al., 2010).

Potassium lactate is a potassium salt of lactic acid (Aymerich et al., 2008; Muchaamba et al., 2021). It is usually used to adjust the acidity, as a preservative in cooked and/or cured meat and poultry products (Aksu & Erdemir, 2021). Potassium lactate is used as a replacement for sodium lactate when there is a necessity to reduce the sodium amount (Muchaamba et al., 2021).

A 1:1 mixture of sodium acetate and acetic acid is commonly used as a preservative, flavoring agent, and pH adjustment in food (Knight et al., 2007). Sodium diacetate is sodium acetate with a slight flavor or taste of acetic acid. It is a salt of acetic acid with a colorless solid state which is used in seasonings and an antimicrobial agent (Drosinos et al., 2006). Sodium diacetate not only exhibits antibacterial activity but also functions as pH controllers, humectants, and flavor in allied meat products (Sohaib et al., 2016).

Drosinos et al. (2006) studied the antimicrobial properties of sodium lactate, sodium acetate, and potassium sorbate on the *Lactobacillus curvatus* in modified MRS broth. Their findings indicated that sodium lactate (2, 3, and 4%), sodium acetate (0.5%), and potassium sorbate (0.15%) combination had the highest antimicrobial activity. Also, results from preservatives addition to two types of thermally processed meats showed that sodium lactate and the combination of sodium lactate, sodium acetate, and potassium sorbate were the most effective, extending the product's shelf life an additional 10 days (Drosinos et al., 2006). Similar results were reported for turkey ham production incorporated with a combination of sodium lactate (2%) and sodium diacetate (0.1%) and also treatment with low-dose irradiation which led to ensuring the safety of RET product against *L. monocytogenes* (Zhu et al., 2005). Muchaamba et al. (2021) reformulated recipes of RTE meat product (salami) aiming to reduce salt content which can mitigate the negative impacts of a high salt diet. Potassium lactate was used as a sodium chloride replacer during salami production. Effects of the high salt (4% NaCl) concentration in standard salami recipes and low salt (2.8% NaCl) plus potassium lactate (1.6%) combination on product characteristics and growth of contaminating *Listeria monocytogenes* were compared. Simulated salami-ripening conditions applied in meat simulation broth and beef indicated that the low salt plus potassium lactate combination retained similar to superior anti-*Listeria* activity compared to the high salt concentration treatment. Salami challenge tests showed that the low NaCl with potassium lactate combination had comparable anti-Listeria activity as the high NaCl concentration during ripening and storage. They concluded that potassium lactate replacement enabled a 30% NaCl reduction without compromising the product quality and antimicrobial benefits of high NaCl concentration inclusion (Muchaamba et al., 2021).

Several studies have declared that organic acids induce some sensorial changes (color and flavor) in meat products. The mechanistic approach of these acids involves that they enter the microbial body, inducing cytoplasmic acidification that can lead to an imbalance of energy. Also, their application results in the accumulation of free acid anions to a toxic level that can kill or at least retard the growth of these microbes (Sohaib et al., 2016). This study aimed to use a combination of potassium lactate and sodium diacetate as preservative agents to extend the shelf life of beef hamburgers regarding the chemical, microbial, and sensorial properties. The oxidative stability, bacterial, mold, and yeast counts, and also sensory acceptability of produced hamburgers were assessed.

2. Material and Methods

2.1. Materials

The chemical materials which were used in the present study were all of analytical grade.

2.2. Preparation of hamburger patties

Beef hamburgers were produced at three different concentrations of minced meat including 30, 70, and 90% according to the method described by Hemmatkhah et al. (2020) and Alanchari et al. (2021). The other components of the hamburgers were salt, spice, toasted flour, wheat flour, and ground onion. Potassium lactate (PL) and sodium diacetate (SD) were also added to the formula by 3% and 0.15%, respectively. The components were homogeneously mixed at a pilot-scale kneader for 5 or 6 min. Then, the mixtures were molded with a diameter of 106 mm, and a thickness of 11 mm reached a weight of 110 g. The prepared hamburgers were stored at -18°C for 48 h for further experiments.

2.3. Determination of chemical properties

Moisture (ISO 1442, 1997), ash (ISO 936, 1998), and protein contents (ISO 937, 1978) of the hamburgers were ascertained according to the international procedures (ISO 1442, 1997; ISO 1978; Perez & Andujar, 1981).

2.4. Oxidation parameters

Two oxidation parameters of hamburger patties were evaluated regarding their different meat contents and also PL and SD addition effects.

2.4.1. Peroxide value (PV)

The PV as the initial indicator of oxidation was determined based on the method described by Rezvankhah et al. (2018). Determination was conducted by titration of 0.1 N KI saturated solutions of the oil with 0.1 N $Na_2S_2O_3$ and starch as an indicator. In brief, the oil of hamburgers was extracted using *n*-hexane and vigorously shaking, then centrifuged to collect the solvent and oil. The solvent was evaporated at a vacuum condition and the obtained oil was used for further analysis. The aliquot of oil was weighed into a 25 mL volumetric flask and 5 mL of chloroform and 10 mL of acetic acid were added to it. The prepared solution was mixed with 1 mL of freshly prepared aqueous KI solution (50% w/v). The mixture was allowed to stand in darkness for 5 min. Then, 30 mL of distilled water and 0.5 mL of starch indicator (0.5% w/v) were added to the mixture and titrated drop-wise with thiosulfate solution until the blue color was disappeared. A blank was analyzed similarly without oil. PV was computed from the equation below:

$$PV = \frac{(S - B) \times M \times 1000}{W} \quad (1)$$

where PV was expressed in mg oxygen equivalent per kg of oil (meq-O_2/kg-oil), S indicated the volume of thiosulfate used for the sample, B indicated the volume of thiosulfate used for blank, M was the molarity of the thiosulfate solution, and W was the weight of oil used for analysis.

2.4.2. Thiobarbituric acid-reactive substance (TBARS)

The TBARS assay was implemented using the method suggested by Peiretti et al. (2012) with brief modification. 10 g of samples were homogenized for 30 s at high speed with 20 mL of 10% trichloroacetic acid (TCA), using a Polytron tissue homogenizer (Type PT 10–35; Kinematica GmbH, Luzern, Switzerland). After centrifugation of the homogenate (600 rpm for 5 min at 4°C), the supernatant was filtered through Whatman #1 filter paper. 3 mL of filtrate was combined with 3 mL of a 0.02 M aqueous 2-thiobarbituric acid solution (TBA), heated in a boiling water bath for 45 min, together with a blank containing 1 mL of a TCA/water mix (1/1) and 1 mL of a TBA reagent, and subsequently cooled under running tap water. The samples were analyzed in triplicate and the results were expressed as mg malonaldehyde (MDA) kg^{-1}, using a standard curve that covered the concentration range of 1-10 mM 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, Steinheim, Germany). The absorbance was read at 532 nm with a Helios spectrophotometer (Unicam Limited, Cambridge, UK) against a blank that contained all the reagents, but no meat (Peiretti et al., 2012).

2.5. Microbial properties

2.5.1. Bacterial total count

The total enumeration of microorganisms was conducted using the method of Sohrabpour et al. (2020). A 1 mL of sterile pipette was used to transfer the initial suspension of hamburger samples and serial dilutions were prepared. Then, 12 mL of the culture medium was added to the plate count agar (Pure Plate). The prepared plates were placed in an incubator at 30°C. After the incubation period, the colonies were enumerated.

2.5.2. Salmonella count

The hamburger samples were combined with 45 mL of sterile 0.1% peptone water (Oxid) and homogenized in a mixing machine for 2 min according to the method of Osaili et al. (2020). To enumerate the survival of *Salmonella* cells, 0.1- or 1-mL aliquots from appropriate decimal dilutions were plated in duplicate on 20-mL portions of Sorbitol MacConkey Agar (Oxid) and Xylose Lysine Deoxycholate Agar (Oxid), overlaid with 10 mL of Tryptone Soy Agar (Oxid) respectively, to resuscitate injured cells from the thermal treatment. After 48 h of incubation at 37°C, typical colonies were enumerated.

2.5.3. Escherichia coli count

The enumeration of *E. coli* O157:H7 in hamburgers enriched with PL and SD was conducted using the Most Probable Number (MPN) method, with tryptic soy broth (Difco, Detroit, MI) as diluent and recovery medium (Chirinos et al., 2002). After 24 h incubation at 37 ºC, tubes showing growth were streaked onto Sorbitol MacConkey agar (Oxoid, England). The colonies were confirmed as *E. coli* O157:H7 using proper biochemical tests and the *E. coli* antiserum O157 assay (Probac do Brasil).

2.5.4. Staphylococcus aureus count

S. aureus was enumerated on Baird Parker Agar (BPA) supplemented with egg yolk tellurite emulsion as a medium after incubation at 37°C for 48 h (Yekta et al., 2020).

2.5.5. Mold and yeast number

Molds and yeasts were enumerated on acidified potato dextrose agar (PDA) used as the culture medium according to the method described by Mohammadi-Gouraji et al. (2019). In brief, 1 mL of the 10^{-1} diluted hamburgers using a Pasteur pipette was dispersed on the surface of sterile PDA. The plates were incubated aerobically upright at 25°C for 72 h.

2.6. Statistical analysis

All experiments were accomplished in three replications and data were provided in mean and standard deviation. Statistical analysis of data was conducted by one-way ANOVA and the mean

difference of data was performed using the Duncan test at the probable level of 5% ($p < 0.05$).

3. Results and Discussion

Hamburger patties with different beef percentages (30, 70, and 90%) were produced and enriched with PL and SD to raise oxidative stability and lower microbial growth. The determined properties of formulated hamburgers have been presented in the following sections.

3.1. Chemical composition

The moisture content of produced hamburgers was determined during 150 days of storage. A declining trend was observed for moisture contents of control (30, 70, and 90% ground beef) and also hamburgers with PL and SD with the same ground beef. Based on Table 1, the control hamburger with 70% ground beef and also the hamburger with 70% ground beef and incorporated SD and PL had relatively higher moisture content compared with other controls and samples. Also, hamburgers containing PL and SD indicated slightly higher moisture content than control attributed to the increase in proteins' water holding capacity. For control and sample hamburgers with 30% ground beef, the moisture content was decreased from 59.26 to 56.73% and 59.63 to 57.66% during the 150 days of storage, respectively. For control and sample hamburgers with 70% ground beef, the moisture content was declined from 63.93 to 58.56% and 54.9 to 59.7%, respectively. For control and sample hamburgers with 90% of ground beef, the moisture content was decreased from 59.7 to 54.73% and 61.03 to 55.86%, respectively. On this basis, using the combination of PL and SD resulted in maintained moisture content during the storage time. PL and SD have been recognized as replacers salts to NaCl. Using PL and SD has been found to reduce the excessive hardening at the surface of salted meat products which leads to efficient drying of product and the moisture would fall more (Aksu & Erdemir, 2021). The same results were obtained for Turkish pastirma cured with potassium lactate and sodium chloride combinations (Aksu & Erdemir, 2021; Erdemir, 2021). Other researchers also reported that sodium lactate replaced with NaCl in rabbit ham and ground beef led to a reduction of moisture content (Blanco-Lizarazo et al., 2015; Tenderis et al., 2021).

Table 1. Moisture content of control hamburgers (containing 30, 70, and 90% ground meat) and hamburgers with added potassium lactate (PL) and sodium diacetate (SD).

Treatment	Storage time (day)				
	1	30	60	90	150
Control (30%)	59.29 ± 0.25	58.30 ± 0.30	57.26 ± 0.15	57.26 ± 0.15	57.66 ± 0.15
Control (70%)	63.93 ± 0.25	62.33 ± 0.55	62.16 ± 0.15	59.73 ± 0.15	58.56 ± 0.35
Control (90%)	59.70 ± 0.30	58.93 ± 0.20	58.13 ± 0.28	56.30 ± 0.26	57.73 ± 0.28
Hamburger (30%) with SD and PL	59.63 ± 0.23	58.96 ± 0.11	57.86 ± 0.35	57.76 ± 0.15	57.66 ± 0.11
Hamburger (70%) with SD and PL	64.90 ± 0.00	63.30 ± 0.62	63.13 ± 0.30	61.00 ± 0.20	59.70 ± 0.34
Hamburger (90%) with SD and PL	61.03 ± 0.28	60.00 ± 0.17	59.10 ± 0.26	57.43 ± 0.28	55.96 ± 0.23

Table 2. Total count of control hamburgers (containing 30, 70, and 90% ground meat) and hamburgers with added potassium lactate (PL) and sodium diacetate (SD).

Treatment	Storage time (day)				
	1	30	60	90	150
Control (30%)	5.43 ± 0.05	5.50 ± 0.02	5.55 ± 0.02	5.62 ± 0.01	5.65 ± 0.00
Control (70%)	5.75 ± 0.00	5.77 ± 0.01	5.80 ± 0.01	5.82 ± 0.01	5.84 ± 0.00
Control (90%)	5.81 ± 0.02	5.81 ± 0.01	5.85 ± 0.00	5.87 ± 0.01	5.91 ± 0.01
Hamburger (30%) with SD and PL	5.26 ± 0.02	5.41 ± 0.06	5.43 ± 0.02	5.53 ± 0.03	5.60 ± 0.00
Hamburger (70%) with SD and PL	5.68 ± 0.02	5.68 ± 0.02	5.73 ± 0.01	5.74 ± 0.02	5.78 ± 0.00
Hamburger (90%) with SD and PL	5.75 ± 0.01	5.75 ± 0.01	5.78 ± 0.01	5.82 ± 0.00	5.84 ± 0.00

Table 3. *E. coli* count of control hamburgers (containing 30, 70, and 90% ground meat) and hamburgers with added potassium lactate (PL) and sodium diacetate (SD).

Treatment	Storage time (day)				
	1	30	60	90	150
Control (30%)	1.62 ± 0.05	1.68 ± 0.04	1.68 ± 0.00	1.73 ± 0.02	1.76 ± 0.00
Control (70%)	1.64 ± 0.02	1.66 ± 0.01	1.71 ± 0.01	1.71 ± 0.01	1.72 ± 0.01
Control (90%)	1.66 ± 0.01	1.70 ± 0.00	1.73 ± 0.00	1.75 ± 0.00	1.78 ± 0.00
Hamburger (30%) with SD and PL	1.45 ± 0.04	1.60 ± 0.01	1.63 ± 0.01	1.66 ± 0.02	1.69 ± 0.01
Hamburger (70%) with SD and PL	1.59 ± 0.01	1.60 ± 0.01	1.65 ± 0.00	1.66 ± 0.02	1.66 ± 0.02
Hamburger (90%) with SD and PL	1.60 ± 0.00	1.65 ± 0.00	1.67 ± 0.00	1.69 ± 0.00	1.71 ± 0.00

3.2. Oxidation

Meat-based products are classified in highly perishable food groups, mainly due to their high water activity, nutritional composition, unsaturated lipids presence, and incorporation of oxygen in the manufacturing which contribute to increased lipid oxidation and microbial load (Vergara et al., 2021).

PV indicates the criterion for first oxidation products' generation including radical peroxyl and also hydroperoxides. When the oxidation is carried on (being accelerated), higher hydroperoxide is formed and thus, PV is increased. However, hydroperoxides are unstable oxidation products that are cleaved and PV would not be more enough confident index for the intensity of oxidation. It would be satisfactory to take advantage of the determination of secondary oxidation products such as aldehydes and ketones continuing the oxidation. Secondary oxidation products are determined by the thiobarbituric acid index (TBARS). It implies the aldehyde and ketones formation which can lead to off-flavor and rancidity.

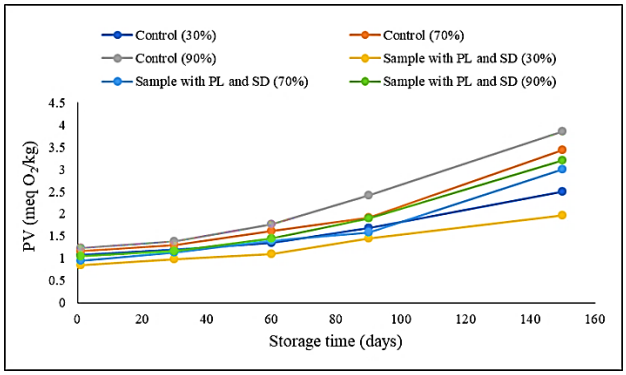

Fig. 1. Peroxide value (PV, meq O_2/kg) of control hamburgers containing 30, 70, and 90% of ground meat and samples with added potassium lactate (PL) and sodium diacetate (SD) with the same ground meat.

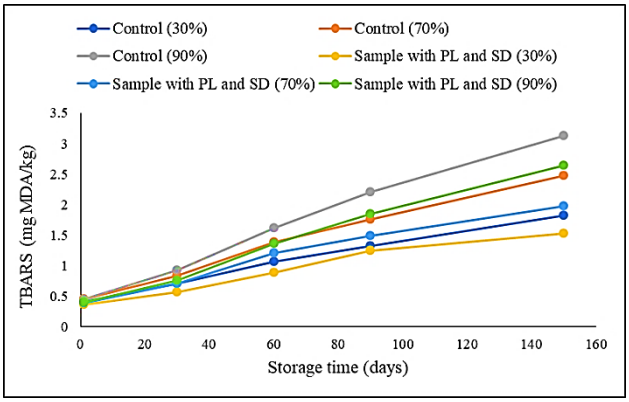

Fig. 2. TBARS values of control hamburgers containing 30, 70, and 90% of ground meat and sample hamburgers combined with potassium lactate (PL) and sodium diacetate (SD).

3.2.1. Initial oxidation stage

PV of hamburger patties was determined during 150 days of storage. The hamburgers were enriched with PL and SD at the 3% and 0.15% concentrations, respectively. According to Fig. 1, it was observed that control hamburgers with no added PL and SD indicated higher PV for all beef concentrations. For control and also main samples, PV was increased with mild steep during 90 days and then increased with the sharper state till 150 days of storage. For control and sample hamburgers with 30% of ground meat, PV was increased from 1.08 to 2.49 meq O_2/kg and 0.85 to 1.97 meq O_2/kg, respectively. For control and sample hamburgers with 70% of ground meat, PV was increased from 1.15 to 3.42 meq O_2/kg and 0.95 to 3 meq O_2/kg, respectively. For control and sample hamburgers with 90% of ground meat, PV was increased from 1.22 to 3.85 meq O_2/kg and 1.04 to 3.20 meq O_2/kg, respectively. On this basis, the hamburger samples with added PL and SD exhibited lower PV associated with reducing or antioxidant effects of salts (Kılıç et al., 2018). The oxidation occurrence in hamburgers is related to the oil used in the formulation or the beef fats. When the higher beef content was used, the oxidation was more intensive. Indeed, the presence of ionic iron in meat (in myoglobin structure) and conferring oxidation states (as prooxidant) led to the oxidation of the oil. Using antioxidants such as PL and SD which participate in reducing chains indicated inhibiting intensive oxidation effects. The same findings have been reported by Kılıç et al. (2018). Zhou et al. (2010) reported that using combined salts such as SD and PL can be more effective than single usage. Aksu and Erdemir (2021) reported that PL addition to ready-to-eat pastırma increased its oxidative stability.

3.2.2. Secondary oxidation stage

Secondary oxidation level is associated with the formation of aldehydes and ketones which lead to off-flavor. The TBARS is indicative of a second oxidation level which can demonstrate the intensity of off-flavor. According to Fig. 2, the TBARS index was increased for control and test hamburgers during 150 days of storage time. However, the addition of PL and SD decreased the oxidation level in test hamburgers. Also, oxidation was more intensive in hamburgers with 90% of ground meat. It could be related to the presence of high content of fat in hamburgers with 90% of ground meat compared to 30 and 70%. The incorporation of PL and SD reduced the oxidation rate via inhibiting the aldehydes and ketones formation cycle. TBARS increased from 0.41 and 0.36 mg MDA kg^{-1} to 1.82 and 1.52 mg MDA kg^{-1} for control and sample hamburgers with 30% of ground meat after 150 days of storage, respectively. For hamburgers with 70% and 90% ground meat, the determined oxidation products (TBARS) were higher than 30% and reached 2.47 and 1.98 mg MDA kg^{-1} and 3.12 and 2.63 mg MDA kg^{-1}, after 150 days of storage, respectively. The same results were obtained for refrigerated sliced salmon enriched by sodium acetate, sodium lactate, and sodium citrate (Ibrahim Sallam, 2007). There are several studies that the addition of lactate and sodium diacetate has led to an increase in the storage of meat-based products (Aksu & Erdemir, 2021; Stella et al., 2014).

Table 4. Mold count of control hamburgers (containing 30, 70, and 90% ground meat) and hamburgers with added potassium lactate (PL) and sodium diacetate (SD).

Treatment	Storage time (day)				
	1	30	60	90	150
Control (30%)	2.00 ± 0.00	2.04 ± 0.03	2.09 ± 0.02	2.07 ± 0.00	2.12 ± 0.01
Control (70%)	2.16 ± 0.11	2.14 ± 0.03	2.17 ± 0.02	2.17 ± 0.02	2.19 ± 0.01
Control (90%)	2.31 ± 0.03	2.32 ± 0.01	2.34 ± 0.05	2.40 ± 0.03	2.49 ± 0.02
Hamburger (30%) with SD and PL	1.69 ± 0.00	1.69 ± 0.00	2.00 ± 0.00	2.01 ± 0.02	2.04 ± 0.04
Hamburger (70%) with SD and PL	2.00 ± 0.00	2.01 ± 0.02	2.06 ± 0.05	2.06 ± 0.02	2.06 ± 0.05
Hamburger (90%) with SD and PL	2.17 ± 0.02	2.19 ± 0.01	2.27 ± 0.04	2.32 ± 0.04	2.37 ± 0.01

Table 5. Yeast count of control hamburgers (containing 30, 70, and 90% ground meat) and hamburgers with added potassium lactate (PL) and sodium diacetate (SD).

Treatment	Storage time (day)				
	1	30	60	90	150
Control (30%)	3.32 ± 0.04	3.39 ± 0.04	3.41 ± 0.02	3.46 ± 0.01	3.47 ± 0.01
Control (70%)	3.42 ± 0.01	3.48 ± 0.03	3.53 ± 0.01	3.54 ± 0.03	3.53 ± 0.03
Control (90%)	3.49 ± 0.02	3.53 ± 0.04	3.53 ± 0.03	3.54 ± 0.01	3.52 ± 0.01
Hamburger (30%) with SD and PL	3.29 ± 0.01	3.35 ±0.04	3.33 ± 0.02	3.36 ± 0.01	3.38 ± 0.07
Hamburger (70%) with SD and PL	3.33 ± 0.03	3.42 ± 0.02	3.42 ± 0.02	3.44 ± 0.02	3.47 ± 0.01
Hamburger (90%) with SD and PL	3.38 ± 0.02	3.46 ± 0.03	3.43 ± 0.03	3.45 ± 0.01	3.45 ± 0.00

3.3. Microbial count

3.3.1. Total count

PL and SD have been also recognized as antimicrobial salts (Aksu & Erdemir, 2021). They can permeate to the cell membrane of bacteria and reduce the water activity of cells leading to plasmolysis (Aksu & Erdemir, 2021; Gassara et al., 2016). According to Table 2, the total count of control and sample hamburgers was increased during storage time. Albeit, this increase was slightly higher for control. The inclusion of PL and SD reduced the microbial growth and thus, the total count was decreased (Aksu & Erdemir, 2021; Aymerich et al., 2008). For control (containing 30% ground meat), the total count was increased from 5.43 to 5.65 log CFU/g while for sample (containing 30% ground meat) combined with PL and SD, the total count was increased from 5.26 to 5.60 during storage. For control with 70 and 90% of ground meat, the total count was increased from 5.75 to 5.84 log CFU/g and 5.81 to 5.91 log CFU/g, respectively. For hamburgers containing 70 and 90% of ground meat and combined with PL and SD, the total count was increased from 5.68 to 5.78 log CFU/g and 5.75 to 5.84 log CFU/g during the storage time, respectively. Therefore, the addition of SD and PL inhibited microbial growth to some extent. The same findings were observed for RTE pastırma, a dry-cured and dried meat product (Aksu & Erdemir, 2021).

3.3.2. Salmonella count

Control and main hamburgers did not show *Salmonella* colony (the test was negative).

3.3.3. E.coli count

According to the results (Table 3), control hamburgers indicated a higher *E.coli* count compared to the hamburgers incorporated with PL and SD. During 150 days of storage, the *E.coli* count of the control hamburger (containing 30% of ground meat) was increased from 1.62 to 1.76 log CFU/g while for the hamburger combined with PL and SD (containing 30% of ground meat), the respective count was increased from 1.45 to 1.69 log CFU/g. For control hamburgers with 70% and 90% of ground meat, the *E.coli* count was increased from 1.64 to 1.72 log CFU/g and 1.66 to 1.78 log CFU/g. respectively. For samples incorporated with PL and SD, the *E.coli* count was increased by 1.59 to 1.66 log CFU/g and 1.60 to 1.71 log CFU/g, respectively for 70% and 90% of ground meat addition. Thus, the addition of mentioned salts could slightly reduce the *E.coli* count.

3.3.4. Staphylococcus aureus count

The *Staphylococcus aureus* count either for control and the sample with PL and SD was lower than 100 numbers.

3.3.5. Mold and yeast count

Mold and yeast counts were enumerated and results were presented in Table 4 and Table 5. Accordingly, based on Table 4, the mold count for the control hamburger with 30% of ground meat was 2.12 log CFU/g after 150 days of storage while it was 2.04 log CFU/g for the hamburger combined with SD and PL. For control hamburgers with 70% and 90%, the mold count was 2.19 and 2.49

log CFU/g, respectively, after 150 days of storage. It was while that for hamburger samples containing 70% and 90% ground beef and incorporated with SD and PL, mold count was reached 2.06 and 2.37 log CFU/g, respectively. Based on results, it was observed that the addition of salts reduced the mold growth possibly related to the reduction of water activity and also plasmolysis effects.

Regarding the yeast count, the same findings were obtained. Based on Table 5, the results showed that the addition of salts could relatively reduce yeast growth.

4. Conclusion

In the present study, new formulations were developed for hamburger using PL and SD as preservatives. Moisture content was maintained when PL and SD were incorporated. The incorporation of these preservatives increased the oxidative stability of hamburgers during 150 days of storage so that the sample with 70% of ground meat had higher oxidative stability (lower PV and TBARS). Also, these salts had both limiting and inhibiting effects on the bacteria, molds, and yeasts. Especially, total count, E. coli, mold, and yeast counts indicated the antimicrobial effects of PL and SD. Generally, these findings suggested that the shelf life of the meat-based food products can be extended by using a combination of storage issues such as freezing and substituting salts.

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Conflict of interest

The authors declared that they have no conflict of interest.

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