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Application of sodium diacetate, potassium lactate and calcium lactate as a microbial decontaminant during processing and storage of the traditional meatballs (rista)

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Abstract

The traditional meat products undergo microbial spoilage and lipid oxidation like other meat products because of their suitability as a medium for microbial growth, which in turn affect safety, quality and shelf life. In the case of traditional meat products like rista, ghostabha, kebab and several other products prepared in Kashmiri wazwan, organic acids or their salts have not been used to date. The present study was therefore aimed to evaluate the effect of different organic acid salts and storage conditions on various quality parameters of traditional meat products. Sodium diacetate (0.25%), potassium lactate (2.5%) and calcium lactate (2.5%) were used in three batches of traditional meat products. Sodium diacetate (SDA) treated samples showed lower total plate count (TPC) under refrigerated storage as compared to ambient storage. The SDA-treated sample retained the lowest value for TBARS (1.9 mg MDA/kg) and free fatty acid (3.2%) on the 15th day of refrigerated storage. Significantly ($P \leq 0.05$) higher values for L*, a* and b* were maintained by SDA treated samples as compared to control, potassium lactate (PL) and calcium lactate (CL) treated samples under refrigeration conditions throughout the storage period. Organic acid treatment improved the quality attributes of the meat product during the storage period. The use of organic acids as a preservative in these products will help in reducing the use of synthetic agents. Further increase in the shelf life of traditional meat products by application of organic acids will help in their commercialization, marketing and round the year availability.

Keywords: Organic acids, Meat products, Total plate count, Free fatty acid, Refrigeration

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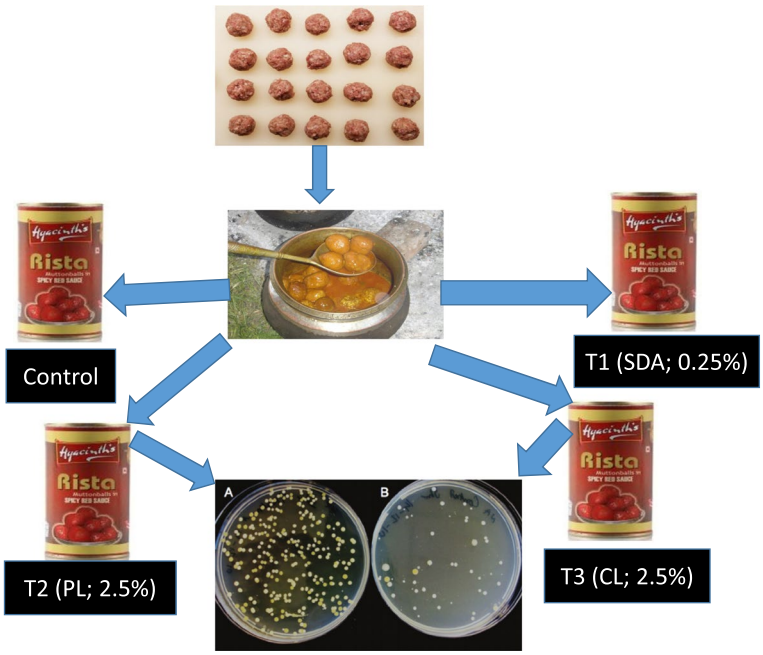
Graphical Abstract



Introduction

Processed meat products are highly cherished by consumers, because of their good taste and convenience. The most common traditional meat products in Jammu and Kashmir, India are rista and ghoshtaba. These are prepared for several occasions like marriage ceremonies, parties, etc. These traditional meat products have great demand because of their distinctive spicy taste and characteristic flavor (Ahmad Mir et al. 2017). Their shelf life is limited because good manufacturing practices (GMP) are not considered during the traditional methods of preparation. These meat products, unless appropriately processed, packaged, transported and stored, spoil in a relatively short time (McMeekin et al. 2006). Microbial contamination is the most critical factor for spoilage, making these products unfit and unsafe for consumption (Brooks et al. 2008). Protein and lipid oxidation are the other negative factors, which lead to the spoilage of such meat products. To curtail all these spoilage-causing agents, different preservatives are added during the processing for the safety and shelf life enhancement of traditional meat products. The incorporation of salts of organic acids and then their storage under refrigeration is an effective way to enhance product storage life with better quality retention. Storage of meat products under refrigeration conditions plays a vital role in lowering oxidation and spoilage (Koutsoumanis & Taoukis 2005). Retention of better quality attributes in meat products under low temperatures has been studied earlier (Koutsoumanis et al. 2006; McMeekin et al. 2006). However, many detrimental changes can still ensue during refrigeration due to microbial growth of psychotropics likes *Pseudomonas* spp. *& Acinetobacter* spp. Besides this other suspected bacterial pathogens of potential concern like *Aeromonashydrophila, Arizona hinshawii, Listeria monocytogenes, Brucella* spp.*& Bacillus anthracis* have been found in different meat products which is a serious concern. The use of salts of organic acids as a preservative is supposed to play a significant role in curbing the growth of spoilage and pathogenic bacteria (Kh I. Sallam & Samejima 2004). Various types of salts of organic acids at different concentrations have been used as a preservative in meat and meat products. Different mechanisms of action for salts of organic acids have been proposed and proven in various meat products, for example, the addition of 2% lactate in pork pate has shown antimicrobial activity, mainly due to the reduction of water activity from 0.959 to 0.945 (Debevere 1989). Potassium lactate has shown bacteriostatic activity, thereby prolonging the shelf life of various meat products (Stekelenburg & Kant-Muermans 2001). The pH lowering effect of sodium diacetate (SD) is responsible for its bactericidal effect and thus helps in the reduction of early bacterial load. Both PL and SD are FDA-approved and classified

as GRAS (Generally Recognized as Safe) ingredients in ready-to-eat (RTE) meat and poultry products. The higher inhibitory effect of calcium lactate against *Listeria monocytogenes* as compared to sodium or potassium lactate during refrigerated storage of pork liver sausage has been studied earlier (Weaver & Shelef 1993). Keeping in view the various above-stated features of salts of organic acids, a need was sensed to use them as preservatives in traditional meat products. Therefore, the main objective of the present study was to evaluate the effect of sodium diacetate (SDA), potassium lactate (PL) and calcium lactate (CL) on physicochemical properties and microbial load in traditional meat products (*rista*) during refrigerated and ambient storage.

Material and methods

Fresh meat (12 kg) from thighs of two sheep (2 years of age, on pasture), slaughtered as per halal method was purchased from a local retail meat shop within 2 h post slaughter at Hazratbal, Srinagar, Jammu and Kashmir, India. Also, 2.5 kg of back fat was procured from the same sheep. The meat and fat were processed within 1 h of procurement for the product development. Spices and other non-meat ingredients required for the product development were also procured from the same market.

Chemicals and reagents

Thiobarbituric acid (TBA), 1,1,3,3-tetra ethoxy propane (TEP) were obtained from Himedia, India. All other chemicals and reagents used in the study were of analytical grade.

Product development

Preparation of the product carried out as per traditional recipe is shown in Table 1. The fat and visible connective tissues of the meat were removed manually with the help of a sharp and clean knife. The lean meat was then ground on the smooth and flat surfaced stone with the help of a wooden hammer. During pounding, spices like cumin (1.5%) and seeds of 3-4 large cardamom were added and pounding was continued for 15 minutes. The fat (20%), which was separately pounded was then added and the pounding was further continued for 10 additional minutes. In the end, salt (2.5%) was added and pounding was done till the meat emulsion of fine consistency was formed. The same practice was repeated till four batches (3 kgs each) of meat emulsions were developed. Meatballs circular in shape with 60 mm diameter and 50-60 g in weight were formed manually from homogenous meat emulsion. These meatballs were then cooked into the gravy for about 25-30 min to get a palatable meat product. The finished product was then filled separately into sterilized glass jars (500 mL) along with the gravy. Four separate batches of filled bottles were created, each comprising 20 bottles to be subjected to treatments. The batch without any treatment was kept as a control. Batch second was treated with sodium diacetate at a concentration of 0.25% and was labeled as SDA, batch third was treated with potassium lactate (2.5%) and labeled as PL and batch fourth was treated with calcium lactate (2.5%) and labeled as CL. Out of 80 bottles developed, 40 bottles were stored at an ambient temperature of 25 ± 1 °C and the rest 40 bottles were refrigerated (4 ± 1 °C) for further storage studies.

Table 1 Product development (g/100 ml) with incorporation of sodium lactate, potassium lactate & calcium lactate

Ingredients	Treatment			
	C	SDA	PL	CL
Raw meat	67.2	67.2	67.2	67.2
Mutton fat	20	20	20	20
Ice water	12.5	9.95	7.7	7.7
Organic salt	0	0.25	2.5	2.5
Cumin	0.1	0.1	0.1	0.1
Common salt	2.5	2.5	2.5	2.5

C, control; SDA, curry with 0.25% sodium diacetate; PL, curry with 2.5% potassium lactate; CL, curry with 2.5% calcium lactate

Microbial analysis

The microbial load of the samples was enumerated at 0, 5, 10, 13 and 15 days of storage according to the methodology described by (Peterkin 1993). A meat sample of 25 g from each batch was homogenized (wise TIS homogenizer HG-15A) with 225 ml of 0.1% peptone water and serial decimal dilution was prepared. Colonies recorded were multiplied with the reciprocal of the dilution and expressed as log10 cfu/g.

Free fatty acid (FFA) estimation

The FFA content of the samples was estimated as per AOAC (2000) method (Horwitz 2000). FFA content was calculated as the percentage of oleic acid by using the following equation:

$$\text{FFA (\%oleic acid)} = \frac{\text{Volume of NaOH(ml)} \times \text{Normality of NaOH} \times \ 28.2}{\text{Weight of fat (g)}}$$

Measurement of lipid oxidation

The method as described by (Andrés et al. 2004) was followed for the estimation of thiobarbituric acid reactive substances which provides a measure of lipid oxidation. TBARS were expressed as mgMDA kg^{-1}meat.

pH

The pH value of the meat samples was determined according to the method described by (Gökalp et al. 2001). The readings were taken in triplicates ($n=3$) from each sample.

Myoglobin denaturation (%)

The myoglobin extraction was carried out according to the method of (Warris 1979). Total myoglobin (Mb) content and percentage myoglobin denaturation (PMD) was calculated using the following formulae (Trout 1989).

$$\text{Myoglobin}\,(\text{mg/ml}) = (\text{A525} - \text{A700}) \times 2.303 \times \text{dilution factor}$$

$$\text{Myoglobin denaturation}(\%) = 1 - \frac{\text{myoglobin concentration after cooking}}{\text{myoglobin concentration before cooking}} \times 100$$

Total volatile base nitrogen (TVB-N)

The method of (Goulas & Kontominas 2005) was used for total volatile base (TVB-N) measurement. The result of TVB-N was expressed as an mg/100 g sample and was calculated from the volume (V) of H_2SO_4 added to it as follows.

$$\text{TVB} - \text{N}(\text{mg}/100\text{g}) = \text{V} \times \text{C} \times 14 \times 100/10$$

Total sulfhydryl content

Total sulfhydryl content was determined by the method of (Eymard et al. 2009), using DTNB as a reagent. Results were expressed in nanomoles of sulfhydryl/mg of protein.

Protein solubility

Sarcoplasmic protein solubility was measured in a low ionic strength solution (150 mM NaCl), as described earlier (Claeys et al. 2002) and was expressed in mg soluble protein/g of meat. Myofibrillar protein solubility was obtained by determining the difference between the total and sarcoplasmic protein solubilities.

Instrumental color analysis

Color variations in control and treated meat samples during storage were examined by evaluating Hunter Lab L* (lightness), a* (redness), and b* (yellowness) values at regular study intervals. Colorimetric analysis on the samples was accomplished by using a hand-held tri-stimulus Color Flex Spectrocolorimeter (COLOR TECH PCM Model: 3001476) with a 25 mm aperture set for illumination D65, 10 standard observer angle. Color readings were measured on randomly selected spots of each sample.

Statistical analysis

The statistical analysis was done by the SPPS software package (SPSS ver. 18; SPSS Inc., Chicago, USA). Data were analyzed by two-way analysis of variance (ANOVA) and the means with SD were compared using Duncan's multiple range test and statistical significance was determined at a 95% confidence level ($P \leq 0.05$).

Result and discussion

Microbial load

The value for the microbial load of the samples as enumerated during the storage period of 15 days is given in Table 2. From the data, it is evident that total plate count (TPC) at refrigerated storage was significantly ($p \leq 0.05$) lower than ambient storage in all samples. There was no growth of microbes till the 5th day of storage however, a TPC of 1.3 log10 cfu/g on the 10th day of storage was recorded, which increased to 5.4 $\log_{10}$ cfu/g on the 15th day of refrigerated storage. During ambient storage, the TPC for the control sample was recorded as 1.6 $\log_{10}$ cfu/g on the 5th day, which on the 15th day of storage was 9.6 $\log_{10}$ cfu/g. Among treatments, sodium diacetate (SDA) treated samples maintained significantly ($p \leq 0.05$) lower TPC as compared to calcium lactate (CL) and potassium lactate (PL) treated samples. This might be due to the higher molecular weight of SDA, with the release of two acetate ions during the dissociation of the salt. The TPC for SDA was 1.9 $\log_{10}$ cfu/g, for PL was 2.5 $\log_{10}$ cfu/g and for CL was 2.1 $\log_{10}$ cfu/g on the 15th day of refrigerated storage. While as, the value of 3.8, 4.7 and 4.1 $\log_{10}$ cfu/g was recorded for SDA, PL and CL treated samples respectively on the 15th day of ambient storage. As found earlier the SL (3%)/SDA (0.25%) combination treatment kept aerobic plate count (APC) levels below spoilage for 70 days on turkey deli loaf slices (Carroll et al. 2007). Similar reports on the inhibition of spoilage microbiota were recorded in different organic acid-treated meat products by (Drosinos et al. 2006) and (Ouattara et al. 1997). *Staphylococcus aureus* was not detected in any samples under refrigerated storage while during ambient storage its growth was recorded from the 10th day in control and the 13th

Table 2 Microbial load (log_{10} cfu/g) of traditional meat product during refrigerated & ambient storage

Conditions	Traits	Treatments	Storage period (days)				
			0	5	10	13	15
Refr.	Total Plate Count	C	ND	ND	1.3 ± 0.23^{c}	2.5 ± 1.21^{bP}	5.4 ± 2.03^{aP}
		SDA	ND	ND	ND	1.1 ± 1.09^{bR}	1.9 ± 1.11^{aR}
		PL	ND	ND	ND	1.7 ± 0.09^{bQ}	2.5 ± 1.05^{aQ}
		CL	ND	ND	ND	1.3 ± 0.03^{bQR}	2.1 ± 0.07^{aQR}
	Staphylococcus aureus	C	ND	ND	ND	ND	ND
		SDA	ND	ND	ND	ND	ND
		PL	ND	ND	ND	ND	ND
		CL	ND	ND	ND	ND	ND
	Yeast & Mold	C	ND	ND	1.6 ± 1.12^{cP}	2.8 ± 0.05^{bP}	4.9 ± 1.01^{aP}
		SDA	ND	ND	ND	1.3 ± 1.19^{bQ}	2.1 ± 2.03^{aR}
		PL	ND	ND	1.2 ± 1.05^{cP}	1.7 ± 1.04^{bQ}	2.7 ± 0.09^{aQ}
		CL	ND	ND	ND	1.5 ± 1.12^{cQ}	2.4 ± 0.04^{aQR}
Amb.	Total Plate Count	C	ND	1.6 ± 0.09^{d}	2.5 ± 1.10^{cP}	3.9 ± 1.08^{bP}	9.6 ± 1.19^{aP}
		SDA	ND	ND	ND	1.8 ± 1.11^{bS}	3.8 ± 1.05^{aS}
		PL	ND	ND	1.7 ± 0.08^{cQ}	2.9 ± 1.09^{bQ}	4.7 ± 0.05^{aQ}
		CL	ND	ND	1.3 ± 1.21^{cQ}	2.3 ± 2.03^{bR}	4.1 ± 0.06^{aR}
	Staphylococcus aureus	C	ND	ND	1.4 ± 0.02^{c}	2.1 ± 0.09^{bP}	5.4 ± 0.18^{aP}
		SDA	ND	ND	ND	1.2 ± 0.19^{bQ}	2.6 ± 0.28^{aR}
		PL	ND	ND	ND	1.5 ± 1.02^{bQ}	3.1 ± 1.19^{aQ}
		CL	ND	ND	ND	1.3 ± 1.13^{bQ}	2.9 ± 0.07^{aQR}
	Yeast & Mold	C	ND	1.8 ± 0.05^{dP}	2.4 ± 1.07^{cP}	3.5 ± 1.09^{bP}	7.5 ± 2.03^{aP}
		SDA	ND	ND	1.1 ± 0.19^{bR}	1.9 ± 1.16^{bR}	2.4 ± 1.13^{aR}
		PL	ND	1.3 ± 0.13^{cQ}	1.6 ± 0.07^{cQ}	2.6 ± 0.47^{bQ}	3.5 ± 2.10^{aQ}
		CL	ND	ND	1.4 ± 1.18^{cQR}	2.1 ± 1.19^{bQR}	2.8 ± 2.08^{abR}

All values are mean ± standard deviation of three replicates ($n = 3$)

Values followed by the same letter in a row and in the column do not differ significantly ($p \leq 0.05$). The letters 'a, b, c, d …' denote differences within a row and 'P, Q, R …' within a column

C: control; SDA: gravy treated with 0.25% sodium diacetate; PL: gravy treated with 2.5% potassium lactate; CL: gravy treated with 2.5% calcium lactate

day in treated samples. The value recorded at the end of ambient storage for control was 5.4 log_{10} cfu/g, for SDA was 2.6 log_{10} cfu/g, PL was 3.1 log_{10} cfu/g and CL was 2.9 log_{10} cfu/g. The lowest value recorded in the case of SDA samples may be due to its increased inhibitory effect at refrigerated temperatures (Islam et al. 2002). Studies have shown that the use of calcium lactate (CL) also enhanced the shelf life of meat products during refrigerated storage (Devatkal & Mendiratta 2001). There was no growth of coliform and *Escherichia coli* in the case of both control and treated samples under refrigerated as well under ambient storage. The absence of *Escherichia coli, Staphylococcus aureus, Salmonella* spp. and *Listeria monocytogenes* could be due to high thermal processing, hygienic practices followed during processing and antibacterial effects of the preservative mixture (Choi & Chin 2003; Geornaras et al. 2006). The yeast and mold count under ambient conditions in control and treated samples were significantly ($p \leq 0.05$) higher than in refrigerated conditions. The yeast and mold count in case of control increased from 1.6 log_{10}cfu/g on the 10th day to 4.9 log_{10}cfu/g on the 15th day of refrigerated storage and from 1.8 log_{10}cfu/g on the 5th day to 7.5 log_{10}cfu/g on the 15th day of ambient storage. In the case of SDA, yeast and mold count increased from 1.3 log_{10} cfu/g on the 13th day to 2.1 log_{10} cfu/g on the 15th day of refrigerated storage. While as, the yeast and mold count for SDA ranged from 1.1 to 2.4 log_{10} cfu/g at the 10th day till the end of ambient storage respectively. The yeast and mold count in the case of PL ranged from 1.2 log_{10} cfu/g on day 10th to 2.7 log_{10} cfu/g on day 15th during refrigerated storage and from 1.3 log_{10} cfu/g on day 5th to 3.7 log_{10} cfu/g at day 15th during the ambient storage. Several studies have used sodium or potassium lactate and sodium diacetate successfully in meat and poultry products to achieve microbiological safety by controlling

foodborne spoilage organisms (Shelef & Addala 1994; Tompkin 2002). Organic acids and their salts showed strong antimicrobial activity, thus helping in the prevention of spoilage of meat products during storage. This has resulted in further enhancement in the shelf life of these meat products, which otherwise are highly perishable and suffer huge losses after their processing.

Free fatty acid (FFA) value

Free fatty acids are the products of enzymatic or microbial degradation of lipids and the determination of FFA gives information about the stability of fat during storage. The effect of organic acid salts and storage time on free fatty acid value reflects in the values given in Table 3. From the data, it is apparent that lower free fatty acid content was recorded under refrigerated conditions as compared to ambient conditions in all samples. However, treated samples under refrigerated conditions have maintained significantly ($p \leq 0.05$) lower values than both treated and control samples under ambient conditions. For the control sample, the free fatty acid value recorded at 0 days of refrigerated conditions was 0.7% which increased to 7.1% on the 15th day of storage, while under ambient storage the value varied from 0.9 to 8.9% from 0 to 15 days of storage period respectively. There was no significant ($p \leq 0.05$) difference in the initial value of free fatty acid among treated samples under refrigerated storage. However, SDA maintained a low value (3.2%) as compared to PL (3.7%) and CL (3.5%) at the end of refrigerated storage. Under ambient storage, SDA treated sample recorded a value of 0.3% at 0 days, which at the end was 4.1%. For PL and CL, the values attained at 0 days were 0.7 and 0.5%, which at the end of storage were 4.6 and 4.3% respectively. Our results are similar with the early findings where lower FFA generation was detected for fish samples treated with sodium acetate as compared to sodium chloride, sodium citrate treated and control sample (Haghparast et al. 2010). The main reason may be due to the slowdown of lipid hydrolysis and the inhibitory effect on microbial growth by the use of sodium acetate in the treated samples. In another study significantly lower FFA content was recorded in buffalo meat nuggets treated with sodium ascorbate (500 ppm) and tocopherol acetate (10 ppm) as compared to control at refrigerated storage (Sahoo & Anjaneyulu 1997).

Free fatty acids are the products of enzymatic or microbial degradation of lipids and the determination of FFA gives information about the stability of fat during storage. In the present study, the organic acids showed significant activity in reducing the formation of free fatty acids during storage. This indicates that the organic acids can act as potential inhibitors of the enzymatic lipolysis, thus will help in the prevention of the production of off-flavors and off odors.

Table 3 Free fatty acid and TBARS value of traditional meat product during refrigerated & ambient storage

Conditions	Parameters	Treatments	Storage period (days)				
			0	5	10	13	15
Refr.	Free fatty acid (%)	C	0.7 ± 0.32^{eP}	2.8 ± 0.16^{dP}	3.6 ± 0.32^{cP}	5.2 ± 0.19^{bP}	7.1 ± 0.49^{aP}
		SDA	0.2 ± 0.13^{eQ}	0.9 ± 0.27^{dR}	1.2 ± 0.19^{cdR}	2.4 ± 0.45^{bR}	3.2 ± 1.29^{aR}
		PL	0.5 ± 0.25^{ePQ}	1.3 ± 0.41^{dQ}	1.8 ± 1.24^{cdQ}	2.9 ± 0.44^{bQ}	3.7 ± 0.21^{aQ}
		CL	0.4 ± 0.31^{ePQ}	1.1 ± 0.32^{dQR}	1.6 ± 0.22^{cdQ}	2.7 ± 0.16^{bQR}	3.5 ± 1.29^{aQR}
	TBARS (mg MDA/kg)	C	0.7 ± 0.22^{dP}	1.6 ± 0.18^{cP}	1.9 ± 0.36^{cP}	2.8 ± 0.47^{bP}	4.1 ± 0.97^{aP}
		SDA	0.1 ± 0.25^{dQ}	0.4 ± 0.42^{cdR}	0.7 ± 0.51^{cR}	1.3 ± 1.21^{bR}	1.9 ± 0.86^{aR}
		PL	0.5 ± 0.19^{dPQ}	0.9 ± 0.28^{cdQ}	1.2 ± 1.71^{cQ}	1.8 ± 0.59^{bQ}	2.6 ± 1.26^{aQ}
		CL	0.3 ± 0.82^{dQ}	0.6 ± 0.76^{cdQR}	0.9 ± 0.51^{cQR}	1.5 ± 0.29^{bQR}	2.2 ± 0.62^{aQR}
Amb.	Free fatty acid (%)	C	0.9 ± 0.39^{eP}	3.6 ± 0.51^{dP}	4.3 ± 0.66^{cP}	6.7 ± 0.81^{bP}	8.9 ± 0.32^{aP}
		SDA	0.3 ± 1.17^{eQ}	1.9 ± 1.49^{dQR}	2.2 ± 0.61^{cR}	3.1 ± 0.44^{bR}	4.1 ± 0.56^{aR}
		PL	0.7 ± 1.29^{eP}	2.1 ± 0.56^{dQ}	2.8 ± 0.78^{cQ}	3.9 ± 0.39^{bQ}	4.6 ± 0.77^{aQ}
		CL	0.5 ± 0.18^{eQP}	1.6 ± 0.21^{dR}	2.4 ± 0.21^{cR}	3.6 ± 0.2^{bRQ}	4.3 ± 0.89^{aQR}
	TBARS (mg MDA/kg)	C	0.9 ± 1.01^{eP}	2.4 ± 0.59^{dP}	3.9 ± 0.44^{cP}	4.8 ± 0.44^{bP}	7.2 ± 0.71^{aP}
		SDA	0.4 ± 0.41^{eQ}	0.8 ± 0.71^{dR}	1.5 ± 0.69^{cS}	2.4 ± 0.82^{bS}	3.3 ± 0.97^{aS}
		PL	0.7 ± 0.52^{ePQ}	1.4 ± 0.87^{dQ}	2.6 ± 0.34^{cQ}	3.2 ± 0.46^{bQ}	4.1 ± 0.89^{aQ}
		CL	0.5 ± 0.43^{eQ}	1.1 ± 1.17^{dQR}	1.9 ± 1.49^{cR}	2.8 ± 0.55^{bR}	3.9 ± 1.16^{aR}

All values are mean ± standard deviation of three replicates ($n = 3$)

Values followed by the same letter in a row and the column do not differ significantly ($p \leq 0.05$). The letters 'a, b, c, d …' denote differences within a row and 'P, Q, R …' within a column

C: control; SDA: gravy treated with 0.25% sodium diacetate; PL: gravy treated with 2.5% potassium lactate; CL: gravy treated with 2.5% calcium lactate

Lipid oxidation

Change in thiobarbituric acid reactive substances (TBARS) value during 15 days of storage at refrigerated and ambient conditions is given in Table 3. Minor changes in the TBARS values were observed in the refrigerated treated samples initially, but values increased on further storage. The lowest TBARS value on the 15th day of storage was observed in SDA treatment, followed by CL treatment and the highest increases in TBARS value was detected in the control sample. The TBARS value recorded for the control sample at the end of refrigerated and ambient storage was 4.1 and 7.2 mg MDA/kg respectively. Among treatments, SDA recorded the lowest value of 1.9 mg MDA/kg, followed by CL (2.2 mg MDA/kg) and PL (2.6 mg MDA/kg) at the end of refrigerated storage. While as, under ambient storage, the TBARS value for SDA was 3.3 mg MDA/kg, for PL was 4.1 mg MDA/kg and for CL was 3.9 mg MDA/kg on the 15th day. Our results are in direct concordance with earlier studies in which the use of sodium lactate used in poultry sausage significantly restricted the increase in the TBARS value (Naveena et al. 2006). Lactate used as a flavoring agent and color stabilizer can also retard lipid oxidation and subsequent off-odor development. In addition, the antioxidant activity of lactate has been stated in pork sausage, chicken sausage and chicken patties (; Naveena et al. 2006). Lactate's role in minimizing lipid oxidation may be due to its ability to scavenge superoxide (O^{2-}) and OH^- radicals. Lipid oxidation is mainly responsible for the quality loss and shelf life reduction of meat and meat products. Various studies have shown that the processing of meat at high temperatures for a longer time duration makes it more prone to lipid oxidation.

pH value

The data about the pH value of the samples is given in Table 4. The pH value of the samples was significantly ($p \leq 0.05$) influenced by the addition of SDA, PL and CL. The increase in pH value in treated samples was lesser during refrigerated storage as compared to ambient storage. In the control sample pH value at 0 days of storage was 5.65, which increased to 6.39 at the end of refrigerated storage. Under ambient storage, the initial pH value was 5.93 while a pH value of 6.82 was recorded on the 15th day of storage. Among treatments, pH value for SDA increased from 4.18 to 5.44, for PL from 4.35 to 5.63 and CL from 4.26 to 5.51 respectively from 0 days to the 15th day of refrigerated storage. While as, pH values varied from 4.35 to 5.95 for SDA, from 4.57 to 6.19 for PL and from 4.43 to 5.83 for CL treated samples respectively from 0 days to the end of ambient storage. As reported earlier the pH value varied from 5.70 and 5.94 by using lactate and acetate salts to increase the shelf life of pork

Table 4 % myoglobin denaturation and pH of traditional meat product during refrigerated & ambient storage

Conditions	Parameters	Treatments	Storage period (days)				
			0	5	10	13	15
Refr.	% myoglobin denaturation	C	48.4 ± 1.22^{eP}	54.3 ± 1.16^{dP}	59.6 ± 0.72^{cP}	64.2 ± 3.19^{bP}	69.9 ± 2.29^{aP}
		SDA	17.7 ± 2.12^{eS}	24.5 ± 2.17^{dS}	36.2 ± 1.19^{cS}	47.6 ± 1.25^{bS}	51.2 ± 1.29^{aS}
		PL	21.9 ± 0.95^{eQ}	29.7 ± 0.38^{dQ}	41.4 ± 0.84^{cQ}	53.8 ± 1.54^{bQ}	58.5 ± 3.31^{aQ}
		CL	19.2 ± 1.21^{eR}	27.9 ± 0.72^{dR}	38.6 ± 0.62^{cR}	49.3 ± 2.06^{bR}	54.9 ± 1.54^{aR}
	pH	C	5.65 ± 0.09^{eP}	5.71 ± 0.02^{dP}	5.83 ± 0.04^{cP}	5.96 ± 0.06^{bP}	6.39 ± 0.09^{aP}
		SDA	4.18 ± 0.02^{eS}	4.33 ± 0.03^{dS}	4.61 ± 0.03^{cS}	4.90 ± 0.04^{bS}	5.44 ± 0.01^{aS}
		PL	4.35 ± 0.03^{eQ}	4.56 ± 0.03^{dQ}	4.78 ± 0.05^{cQ}	5.14 ± 0.03^{bQ}	5.63 ± 0.03^{aQ}
		CL	4.26 ± 0.01^{eR}	4.44 ± 0.01^{dR}	4.70 ± 0.01^{cR}	5.09 ± 0.02^{bR}	5.51 ± 0.04^{aR}
Amb.	% myoglobin denaturation	C	53.0 ± 0.49^{eP}	57.6 ± 0.51^{dP}	65.3 ± 0.36^{cP}	71.7 ± 0.81^{bP}	78.4 ± 0.72^{aP}
		SDA	24.3 ± 1.27^{eS}	33.9 ± 2.49^{dS}	41.6 ± 1.25^{cS}	53.9 ± 1.42^{bS}	61.7 ± 0.92^{aS}
		PL	29.5 ± 1.49^{eQ}	38.2 ± 1.33^{dQ}	47.9 ± 2.28^{cQ}	59.6 ± 1.39^{bQ}	68.1 ± 1.57^{aQ}
		CL	26.8 ± 2.12^{eR}	36.6 ± 2.21^{dR}	45.1 ± 1.36^{cR}	56.9 ± 2.27^{bR}	65.3 ± 2.31^{aR}
	pH	C	5.93 ± 0.08^{eP}	6.11 ± 0.03^{dP}	6.34 ± 0.05^{cP}	6.51 ± 0.02^{bP}	6.82 ± 0.04^{aP}
		SDA	4.35 ± 0.02^{eS}	4.56 ± 0.04^{dS}	4.69 ± 0.03^{cS}	5.23 ± 0.04^{bS}	5.95 ± 0.01^{aR}
		PL	4.57 ± 0.01^{eQ}	4.78 ± 0.03^{dQ}	4.90 ± 0.03^{cQ}	5.67 ± 0.09^{bQ}	6.19 ± 0.02^{aQ}
		CL	4.43 ± 0.03^{eR}	4.64 ± 0.01^{dR}	4.76 ± 0.02^{cR}	5.47 ± 0.05^{bR}	5.83 ± 0.03^{aS}

All values are mean ± standard deviation of three replicates ($n = 3$)

Values followed by the same letter in a row and the column do not differ significantly ($p \leq 0.05$). The letters 'a, b, c, d …' denote differences within a row and 'A, B, C…' within a column

C: control; SDA: gravy treated with 0.25% sodium diacetate; PL: gravy treated with 2.5% potassium lactate; CL: gravy treated with 2.5% calcium lactate

loin (Jensen et al. 2003). An increment in the pH value under cold storage may be due to the production of volatile basic compounds as a result of protein breakdown or bacterial deamination of amino acids with ammonia production. The pH-reducing effect of calcium lactate in meat products was also documented by (Devatkal & Mendiratta 2001). The pH-lowering effect of the use of sodium diacetate (pH 4.3) compared with sodium lactate (pH 7.6) in restructured turkey breast products formulated with a fibrin cold-set binding system, these results are in agreement with our findings (Mohammed Shafit & Williams 2010). Lowering pH and storage of meat at refrigeration temperature have a synergetic effect on the retention of different quality attributes like texture, color and overall palatability of the meat products.

Percent myoglobin denaturation

The value obtained for the percent myoglobin denaturation (PMD) of the samples during study intervals is shown in Table 4. The incorporation of different types of organic acid salts caused a significant ($p \leq 0.05$) impact on the percent of myoglobin denaturation during the storage of the *rista*. Refrigerated conditions have maintained a significantly ($p \leq 0.05$) lower increase in PMD in both control and treated *rista* as compared to ambient conditions. The value of PMD for the control sample at 0 days of refrigerated storage was 48.4%, which at the end of refrigerated storage was 69.9%. In the case of treatments, the value ranged from 17.7 to 51.2% in SDA, 21.9 to 58.9% in PL and 19.2 to 54.9% in CL treated samples respectively at 0 days and 15 days of refrigerated storage. Under ambient conditions, the highest PMD value of 78.4% was recorded in the case of control on the 15th day of storage. SDA-treated samples have recorded a value of 61.7%, followed by CL (65.3%) and PL (68.7%) at the end of ambient storage. A positive relationship between myoglobin denaturation to muscle pH and the color of meat products exists. It was revealed earlier that the undenatured myoglobin of cooked meat products was responsible for the persistent red cooked color, as the pH of fresh meat exceeded 6.0 (Trout 1989). Reduced PMD of high pH meats by the addition of sodium tripolyphosphate was also studied earlier. Succinates are reported to improve myoglobin's resistance to heat-induced denaturation in cooked meat (Zhu & Brewer 2002). Prevention of denaturation of myoglobin by the addition of organic acids/ salts during the storage helps in the retention of better color in processed meat products. Darkening of color or defending of color during the storage results in lower consumer acceptance of the processed meat products.

Total volatile base nitrogen

TVB-N is the most important indicator of spoilage in fresh and lightly preserved muscle foods. The effect of organic acid salts and storage period on the generation of total volatile base nitrogen (TVB-N) is presented in Table 5. From the data, it is apparent that under refrigerated storage the treated samples have retained a significantly ($p \leq 0.05$) lower value of TVB-N as compared to ambient storage. The control sample recorded the highest TVB-N value of 13.38 mg/100 g under ambient storage and a value of 10.57 mg/100 g under refrigeration on the 15th day of storage. Among treatments lowest TVB-N value of 0.36 mg/100 g was recorded for SDA at 0 days, which on the 15th day of refrigerated storage was recorded as 4.94 mg/100 g. While as, the TVB-N value for PL and CL treated samples was 0.64 and 0.93 mg/100 g at 0 days, which on the 15th day were 5.55 and 5.31 mg/100 g respectively. Further, the TVB-N value for PL and CL treated samples ranged from 0.73 to 7.88 and 0.97 to 7.31 respectively 0 days to 15 days of ambient storage. The increase in TVB-N observed during storage might be attributed to the breakdown of nitrogenous substances as a result of microbial activity and any autolytic enzymes found naturally in meat tissue. (Khalafalla et al. 2016) have stated a clear relationship between the microbiological quality of broiler chicken breasts and the level of TVB-N formation. The decrease in the TVB-N value by use of a 2.5% aqueous solution of sodium acetate (NaA) and sodium lactate (NaL) in sliced salmon during storage at 1 °C is in agreement with our studies (Sallam 2007). Total volatile basic nitrogen (TVB-N), which is mainly composed of ammonia and primary, secondary and tertiary amines, gives an early indication of spoilage of meat and meat products. An increase in TVB-N value is related to the activity of endogenous enzymes and spoilage bacteria. TVB-N value increased during storage could explain the pH rising as mentioned above.

Total sulfhydryl content

The microbes while growing produce proteases and lipases which catalyze protein and lipid degradation reactions. Protein oxidation is associated with a decrease in sulfhydryl groups, which are converted into disulfides. The data recorded for total sulfhydryl content during the storage study is presented in Table 5. In samples subjected to different organic acids with different pH values, the treated samples showed significantly lower ($p \leq 0.05$) SH content as compared to the control. This might be due to the protective nature of organic salts of acid against meat protein oxidation. For the control sample, the sulfhydryl content at 0 days was 56.91 nmol/mg protein and at the end of refrigerated storage was 31.25 nmol/mg protein. While as, sulfhydryl content for SDA decreased from 38.21 to 20.48 nmol/mg protein for PL it varied from 44.83 to 25.22 nmol/mg protein and for CL treated sample the value ranged

Table 5 Physicochemical properties of traditional meat product during refrigerated & ambient storage

Conditions	Parameters	Treatments	Storage period (days)				
			0	5	10	13	15
Refr.	TVB-N	C	0.79 ± 1.04^{eQ}	1.48 ± 0.33^{dP}	3.92 ± 1.01^{cP}	5.40 ± 1.13^{bP}	10.57 ± 1.03^{aP}
		SDA	0.36 ± 0.57^{eS}	0.87 ± 0.99^{dS}	1.86 ± 1.61^{cS}	3.68 ± 1.07^{bS}	4.94 ± 1.27^{aS}
		PL	0.64 ± 0.82^{eR}	1.18 ± 1.59^{dQ}	2.27 ± 0.49^{cQ}	3.95 ± 1.58^{bQ}	5.55 ± 0.95^{aQ}
		CL	0.93 ± 1.05^{eP}	1.01 ± 0.44^{dR}	2.11 ± 1.52^{cR}	3.84 ± 2.47^{bR}	5.31 ± 1.07^{aR}
	Total sulfhydryl content	C	56.91 ± 2.73^{aP}	48.14 ± 2.88^{bP}	41.85 ± 1.29^{cP}	38.41 ± 2.67^{dP}	31.25 ± 1.62^{eP}
		SDA	38.21 ± 3.81^{aS}	31.41 ± 1.53^{bS}	28.63 ± 2.14^{cS}	26.07 ± 1.81^{dS}	20.48 ± 2.05^{eS}
		PL	44.83 ± 2.11^{aQ}	36.71 ± 3.04^{bQ}	32.22 ± 1.94^{cQ}	29.29 ± 2.13^{dQ}	25.22 ± 2.18^{eQ}
		CL	42.17 ± 1.84^{aR}	33.05 ± 2.24^{bR}	30.84 ± 2.15^{cR}	27.52 ± 2.72^{dR}	22.63 ± 2.44^{eR}
	Protein solubility	C	68.58 ± 1.03^{aP}	64.35 ± 2.15^{bP}	58.19 ± 1.06^{cP}	53.53 ± 2.21^{dP}	47.71 ± 1.04^{eP}
		SDA	64.65 ± 3.05^{aS}	57.43 ± 3.26^{bS}	50.37 ± 2.17^{cS}	47.95 ± 1.10^{dS}	41.25 ± 1.12^{eS}
		PL	67.93 ± 2.13^{aQ}	63.49 ± 2.18^{bQ}	54.42 ± 1.02^{cQ}	50.01 ± 1.11^{dQ}	45.39 ± 2.16^{eQ}
		CL	66.08 ± 1.10^{aR}	61.64 ± 1.42^{bR}	52.57 ± 2.06^{cR}	49.32 ± 2.10^{dR}	43.98 ± 1.02^{eR}
Amb.	TVB-N	C	0.91 ± 0.35^{eQ}	1.82 ± 0.42^{dP}	4.96 ± 0.14^{cP}	6.83 ± 0.13^{bP}	13.38 ± 0.96^{aP}
		SDA	0.44 ± 0.56^{eS}	1.07 ± 0.55^{dS}	2.17 ± 0.28^{cS}	4.54 ± 1.23^{bS}	6.86 ± 1.22^{aS}
		PL	0.73 ± 0.49^{eR}	1.43 ± 0.42^{dQ}	2.96 ± 0.21^{cQ}	4.97 ± 0.36^{bQ}	7.88 ± 1.16^{aQ}
		CL	0.97 ± 0.54^{eP}	1.21 ± 0.31^{dR}	2.87 ± 0.73^{cR}	4.60 ± 0.85^{bR}	7.31 ± 0.93^{aR}
	Total sulfhydryl content	C	59.16 ± 2.03^{aP}	54.33 ± 2.01^{bP}	51.28 ± 1.33^{cP}	43.24 ± 1.23^{dP}	37.18 ± 0.92^{eP}
		SDA	43.31 ± 0.41^{aR}	41.28 ± 0.62^{bS}	37.23 ± 1.83^{cS}	34.19 ± 3.03^{dS}	29.15 ± 2.05^{eS}
		PL	46.41 ± 3.04^{aQ}	44.36 ± 2.13^{bQ}	40.32 ± 1.44^{cQ}	38.28 ± 1.02^{dQ}	33.24 ± 1.03^{eQ}
		CL	44.12 ± 2.23^{aS}	42.39 ± 3.03^{bR}	39.34 ± 1.24^{cR}	36.30 ± 1.16^{dR}	31.26 ± 0.96^{eR}
	Protein solubility	C	66.34 ± 1.26^{aP}	61.67 ± 1.32^{bP}	54.07 ± 2.08^{cP}	44.54 ± 1.23^{dP}	37.26 ± 1.22^{eP}
		SDA	59.53 ± 2.29^{aS}	54.83 ± 1.24^{bS}	48.96 ± 1.20^{cR}	37.17 ± 1.06^{dS}	30.48 ± 0.96^{eS}
		PL	63.61 ± 1.14^{aQ}	58.13 ± 2.81^{bQ}	51.17 ± 1.53^{cQ}	42.90 ± 1.15^{dQ}	35.71 ± 2.13^{eQ}
		CL	61.21 ± 1.81^{aR}	56.41 ± 1.53^{bR}	46.63 ± 1.87^{cS}	40.07 ± 2.51^{dR}	33.48 ± 2.05^{eR}

All values are mean ± standard deviation of three replicates ($n = 3$)

Values followed by same letter in a row and in the column do not differ significantly ($p \leq 0.05$). The letters 'a, b, c, d …' denote difference within a row and 'P, Q, R …' within a column

C: control; SDA: gravy treated with 0.25% sodium diacetate; PL: gravy treated with 2.5% potassium lactate; CL: gravy treated with 2.5% calcium lactate

from 42.17 to 22.63 nmol/mg protein respectively at 0 days to 15th day of refrigerated storage. Under ambient storage, the highest value of 37.18 nmol/mg protein was recorded for the control sample on the 15th day of storage. The value for SDA increased from 43.31 at 0 days to 29.15 nmol/mg protein on 15 days of ambient storage. A similar decreasing trend in sulfhydryl content was recorded for PL and CL-treated samples. The consequence of such changes is the release of peptides, fatty acids and other decomposition products like sulfhydryl which cause unfavorable changes in meat color, taste and odor. When proteins are targeted by reactive oxygen species, this interaction leads to the generation of carbonyl compounds and the loss of sulfhydryl groups from the protein. A decrease in sulfhydryl content has been reported previously in microsomal membranes from turkey muscle and in minced fish during refrigerated storage (Eymard et al. 2009). The consequence of such changes is the release of peptides, fatty acids and other decomposition products like sulfhydryl which cause unfavorable changes in meat color, taste and odor. When proteins are targeted by reactive oxygen species, this interaction leads to the generation of carbonyl compounds and the loss of sulfhydryl groups from the protein.

Protein solubility

The data about the protein solubility of both control and treatments as obtained during the refrigerated and ambient storage is given in Table 5. There has been a persistent increase in protein solubility with the progression of the storage period. In treatment, the protein solubility was significantly ($p \leq 0.05$) higher under ambient conditions as compared to refrigerated conditions. The value for protein solubility in the case of the control sample ranged from 68.58-47.71 mg/g and 66.34-37.26 mg/g respectively during the 0 to 15 days of refrigerated and ambient storage. In the case of SDA-treated samples, the value of

64.65 mg/g was recorded at 0 days, which at the end of storage was recorded as 41.25 mg/g. While as, value for PL and CL treated samples was 67.93 and 66.08 mg/g at 0 days of storage, which decreased to 45.39 and 43.98 mg/g respectively at the end of refrigerated storage. The lowest value of 30.48 mg/g for SDA, followed by 33.48 mg/g for CL and 35.71 mg/g for PL treated samples was recorded on the 15th day of ambient storage. High values of total protein and sarcoplasmic protein solubility are important for high-quality processed meat products, as they are good indicators of the functional properties of meat products. Our results are in direct agreement with earlier findings wherein the solubility of the proteins was found to increase upon acid marination of sheep and poultry meat (Kim et al. 2006). Various factors like concentration and contents of myofibrillar protein, type and concentration of salts, water holding capacity, pH, processing time and temperature, and additives that interact with each other can affect the protein solubility in cooked meat products. Further, the solubility of protein is a good marker of protein denaturation and is fundamentally related to its hydrophobicity/hydrophilicity balance.

Color value

The Hunter color L^*, a^* and b^* value as recorded during storage study intervals is presented in Table 6. The higher L^*, a^* and b^* values have been retained for the treated samples than for control samples under refrigerated conditions. The L^* value for the control sample decreased from 66.71 to 33.18 during refrigeration and from 56.73 to 15.17 during ambient storage respectively from 0 days to the 15th day of storage. L^* value of 47.13 for SDA, 41.07 for PL and 44.51 for CL was recorded at the end of refrigerated storage, the value of 33.36 for SDA, 25.03 for PL and 29.22 for CL was recorded on the 15th day of ambient storage. The a^* value for the control, SDA, PL and CL ranged from 7.82-3.64, 8.49-5.19, 8.0-4.15 and 8.34-4.77 respectively under the

Table 6 The color value of traditional meat product during refrigerated & ambient storage

Conditions	Parameters	Treatments	Storage period (days)				
			0	5	10	13	15
Refr.	L*	C	66.71 ± 2.16^{aS}	64.05 ± 2.25^{bS}	58.31 ± 1.17^{cS}	50.09 ± 0.78^{dS}	33.18 ± 1.16^{eS}
		SDA	77.50 ± 3.08^{aP}	71.91 ± 1.08^{bP}	66.3 ± 2.09^{cP}	58.8 ± 1.21^{dP}	47.13 ± 0.82^{eP}
		PL	71.11 ± 1.09^{aR}	67.40 ± 2.16^{bR}	60.82 ± 1.42^{cR}	52.41 ± 0.96^{dR}	41.07 ± 1.08^{eR}
		CL	74.32 ± 2.19^{aQ}	69.71 ± 3.09^{bQ}	62.06 ± 1.12^{cQ}	56.70 ± 2.12^{dQ}	44.51 ± 2.17^{eQ}
	a*	C	7.82 ± 1.13^{aS}	7.08 ± 1.04^{bS}	6.33 ± 0.58^{cS}	5.17 ± 0.52^{dS}	3.64 ± 0.19^{eS}
		SDA	8.49 ± 0.21^{aP}	8.17 ± 0.52^{bP}	7.08 ± 1.04^{cP}	6.45 ± 1.12^{dP}	5.19 ± 0.95^{eP}
		PL	8.07 ± 0.61^{aR}	7.25 ± 0.82^{bR}	6.78 ± 1.15^{cQ}	6.03 ± 0.61^{dQ}	4.15 ± 0.16^{eR}
		CL	8.34 ± 0.42^{aQ}	7.63 ± 1.32^{bQ}	6.44 ± 1.17^{cR}	5.90 ± 1.13^{dR}	4.77 ± 0.34^{eQ}
	b*	C	2.92 ± 0.42^{aS}	2.48 ± 1.03^{bS}	1.99 ± 1.03^{cS}	1.55 ± 0.41^{dS}	1.24 ± 0.74^{dS}
		SDA	6.34 ± 1.01^{aP}	6.05 ± 1.25^{bP}	5.61 ± 1.14^{cP}	4.79 ± 1.02^{dP}	4.26 ± 1.08^{eP}
		PL	4.55 ± 0.93^{aR}	4.16 ± 1.02^{bR}	3.73 ± 0.93^{cR}	3.47 ± 1.33^{dR}	3.77 ± 0.41^{eR}
		CL	4.89 ± 1.02^{aQ}	4.51 ± 0.44^{bQ}	4.02 ± 0.62^{cQ}	3.61 ± 0.71^{dQ}	3.95 ± 0.11^{eQ}
Amb.	L*	C	56.73 ± 3.02^{aS}	43.46 ± 2.01^{bS}	31.65 ± 1.43^{cS}	24.05 ± 1.51^{dS}	15.17 ± 1.02^{eS}
		SDA	67.56 ± 1.32^{aP}	62.36 ± 1.03^{bP}	55.51 ± 2.09^{cP}	46.71 ± 2.12^{dP}	33.36 ± 1.18^{eP}
		PL	60.34 ± 1.51^{aR}	56.13 ± 1.64^{bR}	46.70 ± 3.02^{cR}	34.72 ± 1.01^{dR}	25.03 ± 2.51^{eR}
		CL	64.27 ± 2.02^{aQ}	60.92 ± 1.31^{bQ}	52.38 ± 2.03^{cQ}	38.23 ± 2.02^{dQ}	29.22 ± 3.01^{eQ}
	a*	C	6.45 ± 0.41^{aS}	6.01 ± 0.14^{bS}	5.14 ± 1.17^{cS}	4.25 ± 0.45^{dS}	2.64 ± 0.97^{eS}
		SDA	7.33 ± 0.91^{aP}	7.06 ± 0.63^{bP}	6.29 ± 0.42^{cP}	5.28 ± 0.22^{dP}	4.17 ± 1.06^{eP}
		PL	7.07 ± 0.83^{aR}	6.65 ± 1.01^{bR}	5.88 ± 0.69^{cR}	5.04 ± 0.63^{dR}	3.78 ± 1.00^{eR}
		CL	7.21 ± 1.03^{aQ}	6.82 ± 0.79^{bQ}	5.96 ± 0.13^{cQ}	5.15 ± 0.41^{dQ}	3.94 ± 0.93^{eQ}
	b*	C	2.29 ± 1.63^{aS}	2.01 ± 1.18^{bR}	1.56 ± 1.56^{cS}	1.28 ± 0.91^{dS}	1.04 ± 0.45^{eS}
		SDA	6.04 ± 0.52^{aP}	5.81 ± 0.13^{bP}	5.04 ± 0.96^{cR}	4.51 ± 0.47^{dQ}	3.71 ± 0.76^{eP}
		PL	5.86 ± 1.03^{aR}	5.60 ± 0.44^{bQ}	5.17 ± 0.52^{cQ}	4.25 ± 0.25^{dR}	3.27 ± 0.35^{eR}
		CL	5.97 ± 0.63^{aQ}	5.81 ± 0.51^{bP}	5.31 ± 0.32^{cP}	4.62 ± 0.13^{dP}	3.46 ± 0.62^{eQ}

All values are mean ± standard deviation of three replicates ($n = 3$)

Values followed by the same letter in a row and in the column do not differ significantly ($p \leq 0.05$). The letters 'a, b, c, d …' denote differences within a row and 'P, Q, R …' within a column

C: control; SDA: gravy treated with 0.25% sodium diacetate; PL: gravy treated with 2.5% potassium lactate; CL: gravy treated with 2.5% calcium lactate

refrigerated condition from 0 to the 15th day of storage, while under ambient storage the value varied from 6.45-2.64, 7.33-4.17, 7.07-3.78 and 7.21-3.94 respectively at 0 to 15th day of storage. A similar decreasing trend in the b^* values was observed during both refrigerated and ambient in treated as well as control samples. The lowest b^* value of 1.04 for control on the 15th day of ambient storage was recorded, which indicated lesser metmyoglobin reducing activity. However, under refrigerated storage, the b^* value in the case of control decreased from 2.92 at 0 days to 1.24 on the 15th day. The b^* value in the case of SDA, PL and CL treated samples decreased from 6.34 to 4.26, 4.55 to 3.77 and 4.89 to 3.95 respectively from 0 days to the end of refrigerated storage. Under ambient storage, the b^* value for SDA was 6.04 at 0 days, which dropped to 3.71 on the 15th day of storage. For PL and CL treated samples the b^* value decreased from 5.68 to 3.27 and 5.97 to 3.46 at the initial till the end of ambient storage respectively. The lactates have improved color stability in the case of treated samples as compared to the control sample. An increase in color stability could be due to increased metmyoglobin reducing activity (MRA) of mutton meat via NADH produced by lactate dehydrogenase or direct interaction between lactate and myoglobin. Our results are in agreement with previous reports (Kim et al. 2006; Mancini et al. 2009). In another study, it has been reported that potassium lactate darkened meat's appearance and decreased L^* value (Knock et al. 2006). The inclusion of potassium lactate and calcium lactate may have resulted in increased lactate dehydrogenase (LDH) activity, metmyoglobin-reducing activity (MRA) and color stability. This is because lactate addition results in the conversion of lactate to pyruvate by LDH and the generation of NADH. Sodium acetate and diacetate improved color stability in injection-enhanced pork. Color depends on the concentration of myoglobin and the degree of its oxidation, as well as the meat structure. The addition of organic acids /salts and the refrigerated temperature has improved the retention of myoglobin content, reduced microbial spoilage and other quality attributes of the processed meat products.

Conclusions

The present study revealed that treatment with SDA, PL and CL reduced the chemical changes, delayed microbial growth, and improved the color value and sensory attributes of treated rista. Better results in terms of enhancement in protein solubility, decrease in total sulfhydryl content and overall acceptability was attained when rista was treated with SDA as compared to other treatments. It was also observed that the higher L^*, a^* and b^* values have been retained for the treated samples than for control samples under refrigerated conditions. The use of GRAS organic acids and their salts have promising properties that can open new dimensions for the preservation of traditional meat products.

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Authors' contributions
Sajad Ahmad Mir: Original research work and manuscript writing. Shoib Mohd Wani: Editing and review of the manuscript. Zahida Naseem: Review and proofreading. Danish Rizwan: Review and editing. The author(s) read and approved the final manuscript.

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Availability of data and materials
NA

Declarations

Ethics approval and consent to participate
In this study, no animals were involved, hence there was no ethical clearance or approval required.
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Consent for publication
Not applicable.

Competing interests
The authors declare that they don't have any competing interests which could hinder the publication of this article.

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