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Effect of Different Levels of Emulsion pH Adjusted with Lactic Acid and Glucono-Delta-Lactone on the Quality of Pork Sausages

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Abstract: The study was aimed at optimizing the pH hurdle during the process of development of shelf stable pork sausages using hurdle technology. The acidulants used were 0.5 N Lactic Acid (LA) and Glucono-Delta-Lactone (GDL). Different parameters evaluated were pH, emulsion stability, cooking yield, moisture, protein and fat contents, shear force, Lovibond tintometer colour scores, texture profiles and sensory attributes. Reduction in emulsion pH by the addition of LA and/or GDL significantly ($p < 0.05$) influenced the processing and quality parameters of pork sausages. Emulsion pH below 5.90 (i.e., pH of cooked product ~6.00) by the addition of 0.5 N LA affected different sensory attributes adversely. Similarly, incorporation of GDL at or above 0.3% adversely affected most of the quality and sensory attributes of pork sausages. It was observed that the pH of ~5.90 in emulsion achieved by a combination of LA and GDL resulted in better quality characteristics in pork sausages compared to the same pH level attained by either LA or GDL alone.

Key words: Acidulants, pH hurdle, lactic acid, glucono-delta-lactone, quality characteristics, pork sausages

INTRODUCTION

Acidulants are the substances added artificially and deliberately to lower the pH of food, thus extending storage life. Phosphoric acid is the only inorganic acid commonly used in food industry. However, a large number of organic acids such as citric, acetic and lactic acids are now being used as acidulants for a variety of food products. The organic acids in food, either present naturally or added artificially (weak acids), determined particular type of organisms' survival or death, because of their effect on homeostasis and antimicrobial activity. Lowering of cytoplasmic pH is probably the major cause of inhibition of growth by weak acid food preservatives. In many foods, the cytoplasmic pH of microorganisms will normally be one or two units higher than that of their environment (Booth, 1995). If net proton influx cannot be prevented, cytoplasmic pH will fall, leading to cessation of growth and death of the cell (Troller, 1985).

Lactic acid is used as acidifier, pH control agent, curing and pickling agent and inhibitor of microbes (Burdock, 1997). The favorable properties of lactic acid that make it suitable for food applications are a) a mild acid taste in contrast to sharp of most other food acids, b) does not mask or overpower the weaker aromatic flavours of foods, c) has a distinct preservative action and regulates the microflora and d) occurs naturally in many foodstuffs, thus introducing no foreign element to the food. Lactic acid, which is Generally Recognized as Safe (GRAS) is used primarily as acidulant, flavouring and antimicrobial agent. The lactate content with pH of the tissue had a major role in favouring growth of some fermentative gram negative bacteria over that of others and thus alters the

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flora and it was more effective against anaerobic growth than aerobic growth at the ultimate pH (5.8) of beef. The use of lactic acid in foods is limited by good manufacturing practice (Maga and Tu, 1995).

Glucono-Delta-Lactone (GDL) also called D-gluconic acid-delta-lactone, is the cyclic 1,5-intermolecular ester of D-gluconic acid. In cold water, it hydrolyses slowly as an equilibrium mixture of gluconic acid, its delta and gamma lactones. Its aqueous solution thus possesses acidic taste. GDL is used in food industry for its functional properties of being an acidifier, curing/pickling and leavening agent, for pH control and as flavour enhancer (Burdock, 1997). It is a slow release acidulant with a low acidic flavour and good chelating properties. Because of its slow rate of acidification and mild taste characteristics, it finds special applications different from other acidulants. It is much used in cured meats, frankfurters, salami, dessert mixes, bakery mixes, processed cheese, cheese whey-based beverages, fish preserves and other fish products, salted fish pastes, processed or precooked meals, salad dressings etc (Maga and Tu, 1995).

GDL is reported to enhance the preservative action of other preservatives like benzoic acid, sorbic acid and their salts (Berry, 2001). The antimicrobial effect of these preservatives is due to undissociated molecules. The lower the pH, the higher is the number of undissociated molecules. GDL, owing to its low acidic flavour, permits lower pH values to be reached, resulting in the reduction of the amount of preservative agents needed to achieve the effect. GDL has been accorded GRAS status as food ingredient. In this study lactic acid and GDL were evaluated as low pH hurdles to develop shelf stable pork sausages. The objective of the present study was to standardize the optimum level of acidulants (i.e., pH hurdle) for processing shelf stable pork sausages using lactic acid and GDL.

MATERIALS AND METHODS

Lean Pork and Pork Fat

Lean pork and fat (back fat) required for the experiments were obtained from crossbred barrows (75% Landrace × 25% Desi) (60-70 kg live weight) slaughtered as per standard procedure at the experimental abattoir of Livestock Products Technology Division, Indian Veterinary Research Institute, Izatnagar. Meat (about 3 kg in each batch) and fat were obtained from ham portion of the carcass within 0.5 h of slaughter and deboning was done in the processing plant after conditioning in a refrigerator at $4\pm 1^{\circ}\text{C}$ for about 16 h. Additional back fat was obtained from loin portion, if required. Skin, fat and meat were separated manually. Meat was cut into cubes of about 3 cm and ground using 13 mm followed by 8 mm plates in a Seydelmann meat grinder (model WD 114, Stuttgart, Germany). Fat was ground using 13 mm followed by 3 mm plates in the same grinder. Ground meat and fat were packaged in LDPE bags and kept frozen ($-18\pm 1^{\circ}\text{C}$) till subsequent use. Frozen meat and fat were thawed at $4\pm 1^{\circ}\text{C}$ (in refrigerator) for 16 h before use.

Processing of Pork Sausages

Meat emulsion was prepared using Seydelmann food cutter (Model K20 Ras, Stuttgart, Germany) as per the procedure mentioned hereunder with 20% pork fat. About 4 kg batches were made, namely, 2600 g lean pork, 800 g pork fat, 200 g condiments mix and refined wheat flour each, 80 g spices mix and refined salt each and 20 g cane sugar and sodium tripoly phosphate each. Also, sodium nitrite was added at 0.015%. Spice mix was prepared as per the formulation developed in the laboratory. Onion and garlic were used in the ratio 3:1 as condiments. Different ingredients were kept at $4\pm 1^{\circ}\text{C}$ for 1.5 h before chopping, to reduce the temperature rise during emulsification. To the ground lean pork, salt, sugar, sodium nitrite and sodium tripoly phosphate were added and chopped for about 2 min. Condiments mix was then added and chopped again for 2 min (water/ice flakes were not added to reduce the water activity in the sausages). Ground pork fat was slowly incorporated while chopping

which was continued till the fat was completely dispersed in the batter (3-4 min). Spice mix and refined wheat flour were added and chopping was continued for another 1 min to get a fine viscous emulsion. The temperature of the emulsion varied from 10-12°C.

Experiment I

About 4 kg meat emulsion was prepared as per the formulation mentioned above. The emulsion was divided into 5 equal batches. First batch served as control (pH 6.15) while the pH of the remaining 4 batches were adjusted to 5.90, 5.84, 5.78 and 5.71 using 0.5 N lactic acid solution, which was added slowly to the respective emulsions while blending for 1 min in a small Hobart food cutter (Model N-50 G, Troy, Ohio, USA). The reason for selecting mild pH hurdle was that the sour products are least relished by Indians. Meat emulsions were then stuffed into 25 mm diameter cellulose casings (Viskase Nojax, Viskase Co. Inc., Chicago, USA) using hydraulic sausage filler (Mainca, Model EP-25, Spain) and linked manually at about 12 cm intervals. Cooking was done in a steam oven without pressure till the internal temperature reaches 75°C, as recorded by a digital probe thermometer (Model CT-809, Century Instruments (P) Ltd., Chandigarh). The sausages were cooled to room temperature and peeled off the casings.

Experiment II

About 4 kg meat emulsion was prepared as per the formulation mentioned above. The emulsion was divided into 5 equal batches. First batch served as control, while calculated amount of GDL i.e., 0.1, 0.2, 0.3 and 0.4%, respectively dissolved in 10 mL filtered water was added slowly to the remaining 4 batches during blending for 1 min in a small Hobart food cutter (model N-50 G, Troy, Ohio, USA). The additional amount of water added was taken into account while calculating the proximate composition. The emulsions were processed into sausages as mentioned in Experiment I.

Experiment III

About 4 kg of meat emulsion was prepared as per the formulation mentioned above and was divided into 4 treatments. First treatment served as control, while lactic acid (0.5 N) and GDL (dissolved in 10 mL filtered water) were added alone and in combination to the remaining 3 treatments so as to attain a pH of about 5.90 (levels of lactic acid and GDL were selected from previous experiments) as follows:

Treatment I = Control

Treatment II = 0.2% GDL

Treatment III = 0.5 N lactic acid × mL

Treatment IV = 0.1% GDL + 0.5 N lactic acid ×/2 mL

These were added slowly to the respective emulsions during blending for 1 min in a small Hobart food cutter (Model N-50 G, Troy, Ohio, USA). The additional amount of water added was taken into account while calculating the proximate composition. The emulsions were processed into sausages as mentioned in Experiment I.

Analytical Procedures

Physico-Chemical Characteristics

pH was determined using a digital pH meter (Elico, Model LI 127, India). The weight of sausages was recorded before and after initial cooking and the yield was calculated (cooking yield = weight of cooked sausages/weight of raw sausages × 100) and expressed as percentage. The procedure of Kondaiah *et al.* (1985) was followed to measure the emulsion stability. Moisture, fat and protein

contents of the sausages were determined as per standard procedures (AOAC, 1995). The water activity (a_w) of the sausages was measured by Pa_wkit water activity meter (Decagon Devices, USA).

The colour of the cooked pork sausages were measured using a Lovibond Tintometer (Model F, Greenwich, UK). The sample colour was matched by adjusting red (a) and yellow (b) units, while keeping the blue units fixed at 2.0. The corresponding colour units were recorded. The hue and chroma values were determined by using the formula, $(\tan^{-1})^{b/a}$ (Little, 1975) and $(a^2 + b^2)^{1/2}$ (Froehlich *et al.*, 1983), respectively, where a = red unit, b = yellow unit.

Texture Profile Analysis (TPA) of pork sausages was conducted as per the procedure described earlier (Bourne, 1978) using a Stable Microsystems Texturometer (Stable Microsystems Ltd. Surrey, UK) model TA-XT₂ texture analyzer attached to the computer interface. Uniform sized pieces (1.5 cm³) were used as the test samples. They were placed on platform in a fixture and compressed to 50% of their original height at a cross head speed of 50 mm/s through a two cycle sequence, using 25 kg load cell. The texturometer was also used to measure shear force and work of shearing using a Warner-Bratzler blade. Uniform sized samples (1 cm³) were radially sheared with a V-shaped blade attached to plunger at 50 mm/min crosshead speed.

Sensory Evaluation

Standard sensory evaluation method using 8-point descriptive scale (Keeton, 1983) was followed with modifications where 8 = excellent; 1 = extremely poor. The experienced panel (7 members) consisted of scientists and post-graduate students of Division of Livestock Products Technology, IVRI, Izatnagar. Pork sausages were warmed (40-45°C) using microwave oven for 1 min and served to the panelists. The panelists evaluated the samples for appearance, flavour, juiciness, texture, binding and overall acceptability using a standard score sheet. Sensory evaluations were conducted between 3.30-4 pm and filtered tap water was provided to the panelists for rinsing their mouth in between evaluation of different samples.

Statistical Analysis

The experiments were replicated a minimum of three times and the data generated for different quality characteristics were compiled and analyzed using randomized block design. The data were subjected to analysis of variance (one way ANOVA), least significant difference (Snedecor and Cochran, 1995) and Duncan's multiple range tests (Steel and Torris, 1981) for comparing the means to find the effects between treatments for various parameters in different experiments. The smallest difference ($D_{\%}$) for two means to be significantly different ($p < 0.05$) is reported.

RESULTS AND DISCUSSION

Experiment I

Physico-Chemical Characteristics

Emulsion Stability (ES) and Cooking Yield (CY) were decreased significantly ($p < 0.01$) with decrease in pH level (Table 1). The pH of the cooked sausages differed significantly ($p < 0.01$) which were proportional to the emulsion pH. The increased denaturation that could have occurred in muscle proteins at lower pH might be the reason for lower ES and CY encountered in sausages with lower pH values (Papadima and Bloukas, 1999).

Emulsion pH significantly affected ($p < 0.01$) the moisture, protein and fat contents of pork sausages. A significant increase in protein content observed with decrease in pH might be attributed to the higher moisture and fat loss occurred with decrease in pH. It is obvious that decline in the pH of the product approaching the isoelectric point of meat proteins reduce its water and fat binding capacities (Hedrick *et al.*, 1993; Pearson and Gillett, 1997). Barbut (2006) reported that addition of

Table 1: Effect of different levels of emulsion pH adjusted with lactic acid on the quality of pork sausages

Parameters	Emulsion pH				
	Control (6.15)	5.90	5.84	5.78	5.71
Physico-chemical characteristics					
Emulsion stability (%)	97.27±0.02 ^a	95.95±0.02 ^b	94.18±0.03 ^c	93.22±0.02 ^d	86.96±0.02 ^e
Cooking yield (%) [#]	94.37±0.03 ^a	93.90±0.08 ^b	93.20±0.09 ^c	92.12±0.02 ^d	90.09±0.03 ^e
Product pH	6.28±0.03 ^a	6.03±0.02 ^b	5.96±0.03 ^c	5.86±0.02 ^d	5.79±0.03 ^e
Moisture (%)	56.91±0.02 ^a	55.16±0.03 ^b	54.75±0.03 ^c	53.12±0.02 ^d	51.90±0.05 ^e
Protein (%)	16.27±0.04 ^a	17.64±0.03 ^b	17.87±0.01 ^c	18.15±0.01 ^d	18.47±0.02 ^e
Fat (%)	22.62±0.01 ^a	22.14±0.03 ^b	20.99±0.04 ^c	19.86±0.02 ^d	15.79±0.04 ^e
Shear force (N)	14.02±0.06 ^a	12.98±0.15 ^b	12.08±0.04 ^c	10.58±0.02 ^d	9.01±0.06 ^e
Work of shearing (Ns)	49.08±0.21 ^a	45.42±0.51 ^b	42.28±0.15 ^c	37.05±0.09 ^d	31.53±0.23 ^e
Water activity	0.95±0.00	0.95±0.00	0.95±0.00	0.95±0.00	0.95±0.00
Instrumental colour scores					
Redness (a)	3.20±0.01 ^d	3.30±0.01 ^c	3.30±0.01 ^c	3.40±0.01 ^b	4.00±0.00 ^a
Yellowness (b)	6.40±0.01 ^a	5.90±0.00 ^b	5.30±0.03 ^c	4.50±0.10 ^d	4.20±0.12 ^e
Hue	63.43±0.01 ^a	60.81±0.10 ^b	58.15±0.05 ^c	52.85±0.01 ^d	46.40±0.10 ^e
Chroma	7.16±0.01 ^a	6.76±0.08 ^b	6.24±0.02 ^c	5.80±0.01 ^d	5.64±0.07 ^d
Texture profiles					
Hardness (N cm ⁻²)	24.62±0.03 ^a	24.15±0.02 ^a	22.24±0.03 ^b	21.90±0.01 ^c	18.23±0.08 ^d
Adhesiveness (Ns)	-0.15±0.01 ^a	-0.07±0.01 ^b	-0.05±0.03 ^c	-0.05±0.07 ^c	-0.03±0.08 ^d
Springiness (cm)	0.91±0.01 ^a	0.90±0.01 ^b	0.85±0.01 ^b	0.84±0.01 ^b	0.84±0.02 ^b
Cohesiveness (Ratio)	0.54±0.01 ^a	0.52±0.01 ^b	0.44±0.01 ^b	0.43±0.02 ^b	0.39±0.01 ^c
Gumminess (N cm ⁻²)	13.14±0.04 ^a	11.55±0.02 ^b	10.60±0.01 ^c	9.55±0.07 ^d	7.19±0.05 ^e
Chewiness (N cm ⁻¹)	12.09±0.02 ^a	10.44±0.03 ^b	8.93±0.02 ^c	8.12±0.03 ^d	6.16±0.03 ^e
Sensory attributes*					
Appearance	7.00±0.00 ^a	7.00±0.00 ^a	7.00±0.03 ^a	6.80±0.05 ^b	6.67±0.10 ^c
Flavour	7.00±0.00 ^a	7.00±0.00 ^a	7.00±0.00 ^a	6.83±0.05 ^b	6.50±0.09 ^c
Juiciness	7.00±0.00 ^a	6.83±0.02 ^b	6.80±0.06 ^b	6.50±0.10 ^c	6.28±0.10 ^d
Texture	7.00±0.00 ^a	6.83±0.05 ^b	6.83±0.05 ^b	6.50±0.07 ^c	6.27±0.01 ^d
Binding	7.00±0.00 ^a	6.83±0.01 ^b	6.83±0.01 ^b	6.50±0.05 ^c	6.50±0.01 ^c
Overall acceptability	7.00±0.00 ^a	6.83±0.05 ^b	6.80±0.07 ^b	6.50±0.02 ^c	6.28±0.03 ^d

n = 9, [#]n = 3; *n = 21, Based on 8-point descriptive scale; Means±SE with different superscripts in the same row indicate significant difference (p<0.05)

liquid lactic acid caused immediate pH drop and separation of moisture and fat, which in turn resulted in lower CY of salami type products. Pietrasik and Duda (2000) however, observed an inverse relationship between moisture and fat contents, which was attributed to the fat substitution by moisture in low fat products. Shear force and work of shearing of the sausages decreased significantly (p<0.01) with decrease in pH, which may be due to the increased denaturation and subsequent loss in binding properties of meat proteins.

Emulsion pH significantly affected (p<0.01) the Lovibond tintometer colour units of the sausages (Table 1). Redness (a-values) increased, while yellowness (b-values) decreased significantly with decrease in pH. Hue angle and chroma, which measures the saturation of light, were decreased significantly with decrease in pH of the products. This effect was more pronounced in sausages made from emulsions with lower pH values i.e., 5.78 and 5.71. This might be due to pH effect on the myoglobin conversion to metmyoglobin in the cooked meat products (Maca *et al.*, 1999) and concentration of meat pigments due to higher moisture and fat loss that occurred in sausages with lower pH. An increase of a-values and decrease of b-values was observed with decrease of pH in cooked meats (Papadopoulos *et al.*, 1991) and in beef top rounds (Maca *et al.*, 1997). However, Bloukas *et al.* (1977) reported that addition of 2% lactic acid to vacuum packaged low-fat frankfurters did not affect the colour values. A similar finding was also observed in low-fat chicken sausages (Andres *et al.*, 2006).

Texture Profile Analysis (TPA) indicated a significant reduction (p<0.01) in all the textural parameters of the pork sausages with decrease in emulsion pH (Table 1). However, sausages made from emulsion with a pH of 5.90 had comparable hardness, springiness and cohesiveness as that of

control. The pH effect was more distinct in sausages made from emulsion with lower pH i.e., 5.78 and 5.71. The lower springiness values observed with decreasing pH levels might be due to the reduction in elasticity of the products as a result of increased fat loss. Better hardness and cohesiveness values observed in sausages with higher pH might be due to the formation of good quality gel matrix as a result of reduced muscle protein denaturation. Feng and Xiong (2002) observed that the quality of gel matrix had an important role in determining textural properties of cooked frankfurters. Also, a reduction in chewiness, gumminess and hardness in fermented sausages (Gou *et al.*, 1996) and decrease in springiness in salami type products (Barbut, 2005) with decrease in pH was reported.

Sensory Evaluation

pH was found to have significant influence ($p < 0.01$) on the juiciness, texture and binding of the pork sausages (Table 1). Appearance and flavour of the sausages made from emulsions with pH 5.90 and 5.84 were comparable to control, while those of sausages made from emulsions with pH 5.78 and 5.71 were significantly lower ($p < 0.01$) than that of control. Interestingly, it was noted that the significant pH effect observed on colour of the samples in instrumental analysis was not reflected in the sensory evaluation. A non-significant ($p > 0.05$) influence of lactic acid addition at low concentrations on colour of meat products was also reported (Smulders *et al.*, 1986). Juiciness, texture and binding of the sausages made from emulsions with pH 5.90 and 5.84 did not differ significantly ($p > 0.05$) but differ significantly ($p < 0.01$) from all other treatments including control. The better juiciness and texture observed for sausages made from emulsions with higher pH might be due to the better fat and water binding in them as a result of lower denaturation of meat proteins. Karthikeyan *et al.* (2000) observed a linear reduction in flavour, juiciness and texture of goat meat keema with decrease in pH from 5.8 to 5.5. Results indicated that the effect of emulsion pH on sensory attributes was more distinct in sausages made from emulsions with lower pH values i.e., 5.78 and 5.71. The sensory evaluation also indicated that the overall acceptability scores of pork sausages was influenced mostly by their juiciness and texture.

Experiment II

Physico-Chemical Characteristics

Increasing levels of GDL significantly reduced ($p < 0.01$) the pH of emulsion and subsequently in cooked sausages (Table 2). ES and CY were also decreased significantly ($p < 0.01$) with increasing levels of GDL. The significant decrease in moisture and fat levels might have also contributed to the reduction in ES and CY with increase in GDL levels. In other words, at lower pH levels denaturation of muscle proteins might have occurred which resulted in lower ES and CY that observed in sausages with higher GDL levels (Van Roon and Krol, 1985; Papadima and Bloukas, 1999).

Emulsion pH significantly affected ($p < 0.01$) the moisture, protein and fat contents of pork sausages. A significant increase in protein content observed with increase in GDL levels, which might be attributed to the higher moisture and fat loss occurred with decrease in pH values. It is well established that decrease in the pH of the product approaching the isoelectric point of meat proteins reduce its water and fat binding capacities (Hedrick *et al.*, 1993; Pearson and Gillett, 1997). Van Roon and Krol (1985) reported a significant decrease in water binding properties in pork liver and pork loin products, with increasing levels of GDL (0 to 0.5%). Warner-Bratzler shear force and work of shearing were comparable for control and sausages with 0.1% GDL while those of sausages with higher GDL levels decreased significantly ($p < 0.01$).

Increase in GDL levels significantly affected ($p < 0.01$) all the Lovibond-tintometer colour units of the sausages (Table 2). Redness (a-values) increased while yellowness (b-values) decreased with increase in GDL level. Hue angle and chroma of the sausages decreased significantly with reduction in the pH level. This could be due to the effect of GDL on the conversion of myoglobin to metmyoglobin

Table 2: Effect of different levels of emulsion pH adjusted with GDL on the quality of pork sausages

Parameters	GDL level				
	Control	0.1%	0.2%	0.3%	0.4%
Physico-chemical characteristics					
Emulsion pH	6.12±0.01 ^a	6.02±0.01 ^b	5.89±0.03 ^c	5.86±0.01 ^d	5.70±0.01 ^e
Emulsion stability (%)	90.79±0.65 ^a	88.92±0.34 ^b	88.23±0.50 ^b	84.50±0.34 ^c	80.98±0.38 ^d
Cooking yield (%) [#]	93.95±0.29 ^a	91.24±0.25 ^b	89.32±0.24 ^c	87.06±0.32 ^d	85.94±0.19 ^e
Product pH	6.27±0.01 ^a	6.18±0.01 ^b	6.07±0.01 ^c	5.97±0.01 ^d	5.86±0.01 ^e
Moisture (%)	56.88±0.01 ^a	54.37±0.02 ^b	53.61±0.01 ^c	51.80±0.23 ^d	51.28±0.03 ^e
Protein (%)	15.80±0.01 ^d	16.70±0.03 ^c	16.96±0.02 ^c	17.50±0.12 ^b	18.12±0.02 ^a
Fat (%)	24.47±0.07 ^a	22.26±0.03 ^b	21.08±0.03 ^c	18.82±0.07 ^d	15.77±0.02 ^e
Shear force (N)	15.37±0.03 ^a	15.13±0.08 ^a	14.25±0.01 ^b	13.64±0.09 ^b	11.82±0.04 ^c
Work of shearing (Ns)	53.79±0.18 ^a	52.96±0.05 ^a	49.87±0.15 ^b	47.74±0.11 ^b	41.37±0.08 ^c
Water activity	0.95±0.00	0.95±0.00	0.95±0.00	0.95±0.00	0.95±0.00
Instrumental colour scores					
Redness (a)	4.10±0.08 ^d	4.17±0.05 ^c	4.27±0.05 ^b	4.33±0.05 ^a	4.37±0.05 ^a
Yellowness (b)	6.93±0.03 ^a	6.38±0.05 ^b	5.67±0.02 ^c	4.77±0.02 ^d	4.10±0.03 ^e
Hue	59.39±0.33 ^a	56.83±0.18 ^b	53.06±0.01 ^c	47.73±0.03 ^d	43.23±0.13 ^e
Chroma	8.05±0.02 ^a	7.62±0.01 ^b	7.09±0.02 ^c	6.44±0.02 ^d	5.99±0.03 ^e
Texture profiles					
Hardness (N cm ⁻²)	10.440±0.08 ^e	10.070±0.02 ^b	9.050±0.01 ^c	8.830±0.02 ^d	7.260±0.02 ^e
Adhesiveness (Ns)	-0.226±0.01 ^b	-0.035±0.01 ^a	-0.035±0.01 ^a	-0.018±0.01 ^c	-0.007±0.01 ^d
Springiness (cm)	0.866±0.01 ^a	0.864±0.01 ^a	0.853±0.01 ^b	0.784±0.01 ^c	0.709±0.01 ^d
Cohesiveness (Ratio)	0.366±0.01 ^c	0.406±0.01 ^b	0.432±0.01 ^a	0.333±0.02 ^d	0.368±0.02 ^c
Gumminess (N cm ⁻²)	3.800±0.01 ^c	4.060±0.01 ^a	3.930±0.01 ^b	2.970±0.03 ^d	2.660±0.01 ^e
Chewiness (N cm ⁻¹)	3.230±0.01 ^c	3.540±0.01 ^b	3.830±0.02 ^a	2.310±0.01 ^d	1.860±0.01 ^e
Sensory attributes[*]					
Appearance	7.29±0.08 ^a	7.29±0.08 ^a	7.21±0.08 ^a	7.21±0.08 ^a	6.93±0.13 ^b
Flavour	7.21±0.08 ^a	7.21±0.08 ^a	7.00±0.10 ^a	6.71±0.08 ^b	6.43±0.11 ^c
Juiciness	7.21±0.08 ^a	7.21±0.08 ^a	6.93±0.03 ^b	6.71±0.08 ^c	6.21±0.08 ^d
Texture	6.93±0.07 ^a	6.86±0.07 ^a	6.57±0.09 ^b	6.36±0.12 ^c	6.14±0.09 ^c
Binding	6.86±0.05 ^a	6.86±0.05 ^a	6.69±0.07 ^b	6.43±0.09 ^c	6.21±0.08 ^d
Overall acceptability	7.21±0.08 ^a	7.14±0.09 ^a	6.86±0.08 ^b	6.43±0.09 ^c	6.00±0.06 ^d

n = 12, [#]n = 4, ^{*}n = 21. Based on 8-point descriptive scale; Means±SE with different superscripts in the same row indicate significant difference (p<0.05)

in cooked meat products (Maca *et al.*, 1999; Juncher *et al.*, 2000) and higher fat and moisture losses that occurred in sausages at higher GDL levels. Garcia-Zepeda *et al.* (1994) reported that use of 1.5% gluconic acid in ground beef increased redness (a-values) and decreased yellowness (b-values). Similar observations were also reported with decrease in pH values in cooked meats (Papadopoulos *et al.*, 1991) and in beef top rounds (Maca *et al.*, 1997).

Texture profile analysis shown that increase in GDL level in pork sausage formulations had significant effect (p<0.01) on all the textural parameters (Table 2). A significant reduction in hardness was observed in sausages with increase in GDL level. Cohesiveness was significantly higher for sausages with 0.2% GDL, which needs further explanation. Springiness of control and sausages with 0.1% GDL did not differ significantly (p>0.05) while gumminess was higher for sausages containing 0.1% GDL. The effect of GDL on different texture profiles was more distinct with increase in GDL level. A reduction in elasticity as a result of increased fat loss at higher GDL levels might have contributed to the lower springiness values observed in sausages with higher GDL level. The better hardness values observed in sausages with 0.1 and 0.2% GDL might be due to the formation of good quality gel matrix which resulted in reduced muscle protein denaturation. Barbut (2006) reported that decrease in pH in salami type products due to addition of higher levels of GDL resulted in crumbling of meat particles and thereby adversely affected different texture profiles.

Sensory Evaluation

Increasing levels of GDL was found to have marked influence on different sensory attributes of pork sausages and this effect was significant (p<0.01) at higher levels of GDL (Table 2). Panelists

could not differentiate the colour differences that observed in lovibond tintometer except at 0.4% GDL level. Significant reduction ($p < 0.01$) in flavour was observed in sausages containing 0.3 and 0.4% GDL, while texture and binding were significantly decreased even at 0.2% level. The overall acceptability scores were mainly influenced by the juiciness and texture attributes of the products. The better flavour, juiciness and texture scores observed for sausages containing low GDL levels might be due to the better fat and water binding, as a result of lower denaturation of meat proteins at higher pH levels. It was reported that addition of GDL at low levels had no effects on colour of cooked sausages (Duda *et al.*, 1976), cooked ham (Pate *et al.*, 1971) and ground beef (Stivarius *et al.*, 2002). Qvist *et al.* (1994) found that addition of GDL at 0.5% level in bologna type sausages result in off-flavour development. Addition of higher levels ($> 0.5\%$) of GDL was also reported to have adverse effect on texture of salami type products (Barbut, 2006).

Experiment III

Physico-Chemical Characteristics

Addition of 0.5 N LA and GDL alone and in combination decreased the respective emulsion pH significantly ($p < 0.01$) from that of control (pH 6.17) (Table 3). A combination of half the quantities of GDL and 0.5 N LA (Treat IV) also produced the same pH in the emulsion as that obtained when they were added alone in double quantities (Treat II and III). pH of cooked sausages also followed the same pattern that observed in emulsion. ES and CY were reduced significantly ($p < 0.01$) with decrease in emulsion pH. However, it was observed that addition of 0.5 N LA alone to reduce the emulsion pH

Table 3: Effect of different levels of emulsion pH adjusted with LA and GDL on the quality of pork sausages

Parameters	Treatments			
	I (Control) (pH 6.17)	II (GDL) (pH 5.89)	III (LA) (pH 5.90)	IV (GDL+LA) (pH 5.89)
Physico-chemical characteristics				
Emulsion stability (%)	96.52±0.19 ^a	90.91±0.03 ^c	93.18±0.02 ^b	93.00±0.04 ^b
Cooking yield (%) [#]	96.63±0.13 ^a	91.25±0.08 ^c	92.27±0.32 ^{bc}	93.47±0.21 ^b
Product pH	6.26±0.01 ^a	6.05±0.02 ^b	6.09±0.02 ^b	6.07±0.02 ^b
Moisture (%)	56.15±0.07 ^a	53.11±0.07 ^b	53.89±0.02 ^c	53.56±0.01 ^{bc}
Protein (%)	15.56±0.04 ^d	16.26±0.02 ^c	16.92±0.01 ^b	16.89±0.01 ^b
Fat (%)	23.13±0.04 ^a	19.08±0.01 ^d	19.29±0.01 ^c	19.98±0.01 ^b
Shear force (N)	12.42±0.15 ^a	11.07±0.15 ^c	11.12±0.03 ^c	11.62±0.09 ^b
Work of shearing (Ns)	43.48±0.52 ^a	38.76±0.51 ^c	38.93±0.12 ^c	40.68±0.32 ^b
Water activity	0.95±0.00	0.95±0.00	0.95±0.00	0.95±0.00
Instrumental colour scores				
Redness (a)	4.10±0.01 ^c	4.34±0.01 ^b	4.30±0.01 ^b	4.31±0.01 ^b
Yellowness (b)	6.10±0.01 ^a	5.70±0.01 ^b	5.60±0.01 ^c	5.70±0.01 ^b
Hue	45.00±0.00 ^e	53.06±0.00 ^c	52.43±0.05 ^d	53.67±0.01 ^b
Chroma	5.80±0.01 ^d	7.14±0.02 ^b	7.06±0.01 ^c	7.08±0.01 ^c
Texture profiles				
Hardness (N cm ⁻²)	19.53±0.08 ^a	13.72±0.06 ^d	17.86±0.01 ^c	18.41±0.03 ^b
Adhesiveness (Ns)	-0.13±0.01 ^a	-0.01±0.03 ^d	-0.09±0.03 ^b	-0.05±0.01 ^c
Springiness (cm)	0.87±0.07 ^a	0.85±0.01 ^c	0.83±0.08 ^d	0.86±0.07 ^b
Cohesiveness (Ratio)	0.48±0.03 ^a	0.41±0.03 ^d	0.42±0.01 ^c	0.47±0.03 ^b
Gumminess (N cm ⁻²)	8.65±0.02 ^a	5.74±0.01 ^d	7.51±0.02 ^c	8.28±0.01 ^b
Chewiness (N cm ⁻¹)	7.16±0.03 ^a	4.55±0.02 ^c	6.51±0.01 ^b	7.16±0.01 ^a
Sensory attributes[*]				
Appearance	7.00±0.00 ^a	6.25±0.08 ^c	6.50±0.03 ^b	6.50±0.05 ^b
Flavour	7.00±0.00 ^a	6.84±0.02 ^c	6.87±0.01 ^b	6.87±0.05 ^b
Juiciness	6.92±0.03 ^a	6.85±0.05 ^b	6.89±0.03 ^a	6.92±0.03 ^a
Texture	7.07±0.07 ^a	6.61±0.05 ^c	6.73±0.05 ^b	6.78±0.05 ^b
Binding	6.85±0.07 ^a	6.78±0.08 ^b	6.78±0.08 ^b	6.78±0.08 ^b
Overall acceptability	7.07±0.07 ^a	6.78±0.05 ^c	6.78±0.05 ^c	6.98±0.08 ^b

n = 9, # n = 3; *n = 21, Based on 8-point descriptive scale; Means±SE with different superscripts in the same row indicate significant difference ($p < 0.05$)

to ~5.90 (\times mL) and a combination of 0.1% GDL and $\times/2$ mL of LA did not differ in their effect on ES and CY. The addition of 0.2% GDL alone reduced ES and CY further even though it also resulted in an emulsion pH of ~5.90. The denaturation occurred in muscle proteins at lower pH (Papadima and Bloukas, 1999) might be the reason for the lower ES and CY. However, the results indicated that LA caused lower denaturation in meat proteins as compared to GDL.

It was observed that the emulsion pH of 5.90 attained by combination of LA and GDL (Treatment IV) had resulted in better fat retention in sausages than that attained when they were used alone. The higher protein content in treated sausages compared to control might be attributed to the loss of fat and moisture during cooking as a result of decrease in emulsion pH. Warner-Bratzler shear force and work of shearing were significantly decreased ($p < 0.01$) with reduction in emulsion/product pH by the addition of GDL and LA alone or in combination. However, treatment IV had significantly higher shear force and work of shearing than other treatments.

Reduction in pH of the sausages significantly increased ($p < 0.01$) the Lovibond tintometer redness (a-values) while decreased the yellowness (b-values) (Table 3). However, at same pH level, treatments II, III and IV did not differ among themselves for redness (a-values), while treatments II and IV had significantly higher yellowness (b-values) than treatment III. Similarly, at an emulsion pH of ~5.90, hue and chroma were significantly higher for treatment IV and II, respectively. Garcia-Zepedia *et al.* (1994) and Stivarius *et al.* (2002) demonstrated that the combination of gluconic acid and lactic acid at varied rates increased redness (a-values) while reduced yellowness (b-values) in ground beef. Similarly, Juncher *et al.* (2000) shown that addition of 2% lactic acid + 0.25% GDL in cooked cured emulsion type meat product improved the red colour (a-values) compared to the control which did not contain LA or GDL.

Texture profile analysis revealed that reduction in pH of emulsion by the addition of LA and GDL alone or in combination significantly reduced ($p < 0.01$) different textural attributes of the sausages (Table 3). Even at same emulsion pH of ~5.90, treatment IV had significantly higher hardness than that of treatments II and III. Similarly, treatment IV had significantly higher springiness, cohesiveness, gumminess and chewiness values than those of treatment II and III. More interestingly, the different textural attributes of sausages from treatment IV were very close to those of control sausages which clearly suggest that addition of a combination of LA and GDL is better than adding them individually in pork sausages. It is obvious that decrease in the pH of product near to the isoelectric point of meat proteins induces more denaturation and thereby adversely affect the textural properties in meat products (Hedrick *et al.*, 1993; Pearson and Gillett, 1997). A reduction in elasticity, as a result of increased fat loss at lower pH levels might have contributed to the lower springiness values observed in sausages made from treatments II to IV. Barbut (2006) also reported similar observations in salami type meat products, where he found that reduction in pH by addition of liquid LA and GDL resulted in crumbling of meat particles and thereby adversely affected different textural attributes of the product.

Sensory Evaluation

Addition of LA and GDL alone or in combination to decrease the emulsion pH significantly reduced most of the sensory attributes of the pork sausages (Table 3). Sausages from treatments III and IV had better appearance than those from treatment II. At same emulsion pH of ~5.90, panelists preferred the appearance, flavour and texture of sausages made from treatments III and IV compared to those from treatment II. Panelists observed similar juiciness in sausages from treatments III, IV and control. The better flavour, juiciness and texture observed in treatments III and IV than treatment II might be attributed to better fat and water binding as a result of lower denaturation of meat proteins. Overall acceptability scores were significantly higher ($p < 0.01$) for sausages made from treatment IV compared to those from treatment II and III which could be attributed mostly to their better juiciness and textural scores. Karthikeyan *et al.* (2000) reported a linear reduction in flavour, juiciness and texture of goat meat keema as the pH was reduced from 5.8 to 5.5 using GDL and LA. Addition of higher

levels of GDL and LA was reported to have adverse effect on texture of salami-type meat products (Barbut, 2006). However, Juncher *et al.* (2000) observed that addition of 2% lactic acid + 0.25% GDL did not significantly affected different sensory attributes of cooked cured emulsion type meat products.

CONCLUSIONS

Reduction of emulsion pH by adding GDL or LA alone and in combinations significantly decreased the processing and quality characteristics of pork sausages. Reduction of emulsion pH below 5.84 (pH of cooked product ~6.00) affected different sensory attributes adversely. Moreover, panelists expressed a sort of sourness in products made from emulsions with pH 5.78 and 5.71. Sensory evaluation indicated that GDL can be incorporated in pork sausages up to 0.2%. The additional advantage of using GDL with LA alone as with LA alone sausages showed a similar quality characteristics with that of combination (treatment IV). The pH of ~5.90 in emulsion achieved by combination of LA and GDL resulted in better quality characteristics, especially product yield and shear force, in sausages compared to the same pH level attained by either LA or GDL alone.

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REFERENCES

- Andres, S.C., M.E. Garcia, N.E. Zaritzky and A.N. Califano, 2006. Storage stability of low-fat chicken sausages. *J. Food Eng.*, 72: 311-319.
- AOAC, 1995. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington DC.
- Barbut, S., 2005. Effect of chemical acidification and microbial fermentation on the rheological properties of meat products. *Meat Sci.*, 71: 397-401.
- Barbut, S., 2006. Fermentation and chemical acidification of salami-type products. Effect on yield, texture and micro structure. *J. Muscle Foods*, 17: 34-42.
- Berry, S.K., 2001. Role of acidulants in food industry. *J. Food Sci. Technol.*, 38: 93-104.
- Bloukas, J.G., E.D. Paneras and G.C. Fournitzis, 1977. Sodium lactate and protective culture effects on quality characteristics and shelf-life of low-fat frankfurters produced with olive oil. *Meat Sci.*, 45: 223-238.
- Booth, I.R., 1995. Regulation of cytoplasmic pH in bacteria. *Microbial Rev.*, 49: 359-378.
- Bourne, M.C., 1978. Texture profile analysis. *J. Food Sci.*, 32: 62-67.
- Burdock, G.A., 1997. Encyclopedia of Food and Colour Additives, Vol. I-IV, CRC Press Inc., New York.
- Duda, Z., J. Mielnik, A. Nowak and W. Pezacki, 1976. Effects of glucono-delta-lactone on the colour and colour stability of cooked comminuted sausages. *Proceeding European Meeting of Meat Research Workers*, 22: 1-8.
- Feng, J. and Y.L. Xiong, 2002. Interactions and functionality of mixed myofibrillar and enzyme-hydrolyzed soy proteins. *J. Food Sci.*, 67: 2851-2856.
- Froehlich, D.A., E.A. Gullet and W.R. Osborne, 1983. Effect of nitrite and salt on the colour, flavour and overall acceptability of ham. *J. Food Sci.*, 48: 152-154.
- Garcia-Zepeda, C.M., C.L. Kastner, B.L. Willard, R.K. Phebus, J.R. Schewnke, B.A. Fijal and R.K. Prasai, 1994. Gluconic acid as a fresh beef decontaminant. *J. Food Prot.*, 57: 956-962.

- Gou, P., L. Guerrero, J. Gelabert and J. Arnau, 1996. Potassium chloride, potassium lactate and glycine as sodium chloride substitutes in fermented sausages and in dry cured pork loin. *Meat Sci.*, 42: 37-48.
- Hedrick, H.B., E.D. Aberle, J.C. Forrest, M.D. Judge and R.A. Merkel, 1993. Principles of Meat Science. 3rd Edn., Kendall/Hunt Publishing Co., Dubuque, Iowa, pp: 133-142.
- Juncher, D., C.S. Vestergaard, J. Soltfoft-Jensen, C.J. Weber, G. Bertelsen and L.H. Skibsted, 2000. Effects of chemical hurdles on microbiological and oxidative stability of a cooked cured emulsion type meat product. *Meat Sci.*, 55: 483-491.
- Karthikeyan, J., S. Kumar, A.S.R. Anjaneyulu and K.H. Rao, 2000. Application of hurdle technology for the development of caprine keema and its stability at ambient temperature. *Meat Sci.*, 54: 9-15.
- Keeton, J.T., 1983. Effect of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. *J. Food Sci.*, 48: 879-881.
- Kondaiah, N., A.S.R. Anjaneyulu, N. Sharma and H.B. Joshi, 1985. Effect of salt and phosphate on the quality of buffalo and goat meat. *Meat Sci.*, 15: 183-192.
- Little, A.C., 1975. Off on a tangent. *J. Food Sci.*, 40: 410-412.
- Maca, J.V., R.K. Miller, M.E. Bigner, L.M. Lucia and G.R. Acuff, 1999. Sodium lactate and storage temperature effects on shelf life of vacuum packaged beef top rounds. *Meat Sci.*, 53: 23-29.
- Maca, J.V., R.K. Miller, J.D. Maca and G.R. Acuff, 1997. Microbiological, sensory and chemical characteristics of vacuum packaged cooked beef top rounds treated with sodium lactate and sodium propionate. *J. Food Sci.*, 62: 586-590.
- Maga, J.A. and A.T. Tu, 1995. Food Additive Toxicology. Mario Dekker Inc., New York.
- Papadima, S.N. and J.G. Bloukas, 1999. Effect of fat level and storage conditions on quality characteristics of traditional Greek sausages. *Meat Sci.*, 51: 103-113.
- Papadopoulos, L.S., R.K. Miller, L.J. Ringer and H.R. Cross, 1991. Sodium lactate effect on sensory characteristics, cooked meat colour and chemical composition. *J. Food Sci.*, 56: 621-626.
- Pate, T.D., R.O. Shuller and R.W. Mandigo, 1971. The influence of glucono delta lactone on cured ham colour and colour stability. *J. Food Sci.*, 36: 48-50.
- Pearson, A.M. and T.A. Gillett, 1997. Processed Meats. 3rd Edn., CBS Publishers and Distributors, New Delhi, pp: 53-77.
- Pietrasik, A. and Z. Duda, 2000. Effect of fat content and soy protein/carrageenan mix on the quality characteristics of comminuted, scalded sausages. *Meat Sci.*, 56: 181-188.
- Qvist, S., K. Schested and P. Zeuthen, 1994. Growth suppression of *L. monocytogenes* in meat product. *Int. J. Food Microbiol.*, 24: 283-293.
- Smulders, F.J.M., P. Barendsen, J.G. VanLogtesty, D.A.A. Mossel and G.M. Van der Marel, 1986. Lactic acid: Considerations in favour of its acceptance as meat decontaminant. *J. Food Technol.*, 21: 419-436.
- Snedecor, G.W. and W.G. Cochran, 1995. Statistical Methods. 8th Edn., Oxford and IBH Publishing Co., New Delhi.
- Steel, R.G.D. and J.H. Torris, 1981. Principles and Procedures of Statistics : A Biometrical Approach. Ch. 8. 2nd Edn., McGraw Hill International Book Co, pp: 173-175.
- Stivarius, M.R., F.W. Pohlman, K.S. McElyea and J.K. Apple, 2002. The effects of acetic acid, gluconic acid and trisodium citrate treatment of beef trimmings on microbial, colour and odour characteristics of ground beef through simulated retail display. *Meat Sci.*, 60: 245-252.
- Troller, I.A., 1985. Effects of a_w and pH on Growth and Survival of *S. aureus*. In: Properties of Water in Foods. Simatos, D. and J.L. Multon (Eds.), Martinus Nijhoff Publishing Co, Dordrecht. pp: 247-275.
- Van Roon, P.S. and B. Krol, 1985. A factorial analysis of water-binding properties and firmness of heated, comminuted pork liver and pork loin products as influenced by addition of sodium chloride and pH regulators. *Meat Sci.*, 13: 65-80.