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ARTICLE

# Effect of GdL Addition on Physico-chemical Properties of Fermented Sausages during Ripening

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**Abstract**

This study investigated the effects of glucono-δ-lactone (GdL) addition on physicochemical and microbiological characteristics of fermented sausages during ripening and drying. Five batches of sausages were produced under ripening conditions: without GdL and with 0, 0.1, 0.25, 0.5 and 0.75% of GdL addition. Samples from each treatment were taken for physicochemical and microbiological analyses on the 0, 1, 3, 5, 7, 10, 15, 20 and 25th day of ripening. Chemical analysis showed a significant decrease in moisture content of sausages with increasing amounts of GdL added ($p$<0.05). The moisture contents decreased, whereas the fat, protein and ash contents increased throughout ripening ($p$<0.05). Increasing levels of GdL caused a decrease in the pH values ($p$<0.05), which can have an inhibitory effect against microflora. Water holding capacity content of samples decreased with increasing GdL concentration ($p$<0.05). The shear force values of fermented sausages showed the highest in T4 ($p$<0.05). During ripening, the shear force values of sausages were increased on the 25th day compared to day 0 ($p$<0.05). The higher GdL level produced lighter and more yellow sausages. The addition of 0.75% GdL was effective in controlling bacteria counts. Addition of GdL in sausages resulted in the physicochemical and microbiological attributes equal to or better than no addition of GdL without any harmful effect.

**Keywords:** fermented sausages, physico-chemical trait, bacteria counts

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## Introduction

Fermented sausages are the result of biochemical, microbiological, physical and sensorial changes occurring in a meat mixture during ripening. During sausage fermentation, complex physicochemical reactions in sausages take place that result in a decrease in pH and changes in the initial microflora (Casaburi *et al*., 2007). Sausages are produced from a meat batter composed of lean and fat meat, and other ingredients (curing agents etc.), stuffed in natural or synthetic casings, and, then, subjected to a ripening process under controlled temperature and relative humidity (Coloretti *et al*., 2014). In addition to meat, many other ingredients can be added to the batter, including spices (black and red peppers, fennel, nutmeg, cumin, etc.), curing agents (nitrate and nitrite), sodium chloride, sugars (to favor lactic fermentation) and starter cultures (lactic acid bacteria, micrococci, staphylococci and fungi) (Toldrá, 2006).

GdL (glucono delta lactone) is a Generally Recognized as Safe (GRAS) substance and is a weak acid, which converts to gluconic acid in water and slowly dissociates into hydrogen ions with time (Chang *et al*., 2009). After all, GdL slowly hydrolyzes to gluconic acid with a resulting reduction in pH, which finally causes the residual nitrite reduction (Juncher *et al*. 2000). Also, GdL as an acidulant has been widely used in acidified food products to improve product texture (Tseng and Xiong, 2009) and it can contribute the formation of meat myofibrillar protein gels by slow dialysis of acid (Ngapo *et al*., 1996). The prevalent usage of GdL in thermal-processed foods would avoid some of the difficulties related with bacterial contamination and develop the product texture without any health problem (Juncher *et al.,* 2000; Lemay *et al*., 2000). In meat products, GdL are used as not only lowering the pH of the fermented sausages such as salami, but controlling bacterial contamination (Bohmc *et al*., 1996). Little is known about the addition of GdL at different concentrations on physicochemical and microbiological traits of the fermented sausages during ripening. Therefore, the aim of this study was to evaluate the effect of GdL addition at

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different concentrations on physicochemical and microbiological characteristics of fermented sausages during ripening.

## Materials and Methods

### Fermented sausages manufacture

For fermented sausages production, fresh beef and pork, and frozen pork backfat were obtained in a vacuum packaged condition from a local meat packer. Pork and beef meat were trimmed of visible fat and pork backfat of adhering skin. Both meats and pork backfat were cut and weighed in appropriate amounts, vacuum packed and kept frozen at -24ºC for 3 d. Five treatments of fermented sausages were prepared at different levels (0, 0.1, 0.25, 0.5 and 0.75%) of GdL. Fermented sausages were made with ground beef, pork, pork backfat, sun-dried salt (2.8%), garlic (0.05%), mettwurst additive (0.1%), monosodium glutamate (0.05%), glucose (0.4%) and starter culture (0.4%). A commercially available frozen meat starter culture (Lyocarni RBL-73, SACCO, Italy) consisting of *Lactobacillus curvatus* and *Staphylococcus xylosus* with $2.5\times 10^6$ Log CFU/g was added at a concentration of ca. 6 Log CFU/g, according to the manufacturer's instructions. Beef, pork and backfat were cut in a bowl chopper (Fujee Co., Korea) at low speed to the desired particle size, about 3-5 mm and mixed in a mixer (Fujee Co., Korea). Starter culture and ingredients were added during mixing. The sausages were produced with GdL at five levels (0, 0.1, 0.25, 0.5 and 0.75%). All treatments, about 10 kg each, were replicated three times in separate meat sources of each replicate. The mixture was then stuffed using a stuffer (H20E, TALSA Co., Northampton, EU) into 55 mm diameter fibrous casings (Korea). The casings had been presoaked in lukewarm water. Sausage filling was restrained and tied with a string by hand. Special cleaning and sanitizing measures were taken during sausage manufacture in a pilot plant. All equipment was rinsed thoroughly between treatments. Samples were dried and ripened in a laboratory smoke chamber (BTDS76P, Bradley, USA). The following conditions of relative humidity (RH) and temperature were applied: day 0 until day 3, 93% RH and 19 ±1°C; day 4 until day 7, 88-90% RH and 18±2°C; day 7 until day 10, 87% RH and 16°C; after day 10, 80% RH and 16°C. The sausages were smoked after 8 and 21 d in the chamber. Samples from each treatment were taken for physicochemical and microbiological analyses on the 0, 1, 3, 5, 7, 10, 15, 20 and 25th d of ripening. All the results in proximate composition were expressed as the mean of triplicate trial at each sampling time. Experimental data in physicochemical traits were reported as mean values with the corresponding standard errors of the mean (SEM) of replicates.

### Proximate composition and physico-chemical analyses

Before analysis, the fat was manually removed from the sausages slices by a knife. All determinations were carried out on the homogenized sample, in triplicate. Moisture, fat, protein and ash were determined on samples using with a slightly modified method of AOAC (2000) on the 0, 5, 10, 15, 20 and 25th d of ripening. The pH of samples was determined with a pH meter (PHM 201, Radiometer, France). The pH values of samples were measured by blending a 10 g sample with 90 mL distilled water for 60 s in a homogenizer (Ultra-turrax, T25-S1, Germany). The water holding capacity (WHC) was conducted by a modification of the procedure of Grau and Hamm (1953). Briefly, a 300 mg sample of muscle was placed in a filter-press device and compressed for 2 min. WHC was calculated from duplicate samples as a ratio of the meat film area to the total area; hence, a larger value suggests a higher WHC. WHC (%) was calculated as follows: WHC (%) = 100 − [total meat area / meat film area × 100]. Shear force values were analyzed by the method described by the procedure of Bourne (1978). The samples were prepared a cubic form (30×30×20 mm) and were cut perpendicular to the longitudinal orientation of the muscle fiber with a Warner-Bratzler shear attachment on a texture analyzer (TA-XT2, Stable Micro System Ltd., U.K.). The maximum shear force value (kg) was recorded for each sample. Test and post-test speeds were set at 1.0 mm/s. For measurement of volatile basic nitrogen (VBN), a micro-diffusion method described by Conway (1950) was modified for the determination of VBN in sausage samples. Each sample (3 g) was homogenized (Ultra-turrax, T25-S1, IKA, Germany) for 30 s with 27 mL of distilled water. The supernatant solution was filtered using a filter paper (No.4, Whatman). A 0.01 N of boric acid was placed in the inner section of a Conway micro-diffusion cell (SibataLtd, Japan). A 1 mL sample solution and 1 mL of saturated $K_2CO_3$ were also placed into the outer section of the same cell, and the lid was immediately closed. The cell was incubated at 25°C for 60 min, and it was then titrated against 0.02 N $H_2SO_4$. The VBN value was reported as mg%. The TBARS of samples were analyzed by the modification method described by the procedure of Witte (1970). Readings were made on a spectro-

photometer (X-MA 3000, Human Ltd., Korea) at 530 nm. Color measurements were taken with a Minolta chromameter (Model CR-410, Minolta Co. Ltd., Japan). CIE L*, a* and b* values were determined with measurements standardized with respect to a white calibration plate (L*=94.4, a*=0.313, b*=0.319) after 30 min blooming at room temperature. Color measurements for each of three replicates, always trying to avoid area with excess fat were taken and the value was recorded.

**Microbiological analysis**

Ten grams of samples from each treatment was also weighed and then homogenized with 90 mL distilled water using a stomacher (STOMACHER® 400 CIRCULATOR, Seward, Ltd., UK) for 90 sec at room temperature. Total aerobic plate counts (TACs) were analyzed according to the Standards for Processing and Ingredients Specifications of Livestock Products, Animal, Plant and Fisheries Quarantine and Inspection Agency Notification (QIA, 2014). Homogenized microbial extracts were serially diluted with distilled water by 10-fold. Portions of the samples (0.1 mL) were plated separately on each plate and spread thoroughly. TACs were enumerated on plate count agar (DifcoTM, Laboratories, USA) and colonies were counted after incubation at 35±1°C for 48 h. *Pseudomonas* spp. were assessed by spread technique on *Pseudomonas* Agar (DifcoTM, Laboratories, USA), incubation at 30±1°C for 48 h. All analyses were performed in duplicate, and results expressed as logarithm colony-forming units per gram of samples (Log CFU/g).

**Statistical methods**

The effect of the GdL treatments on the physicochemical and microbiological analysis of fermented sausages during ripening was analyzed by two-way factor factorial analysis. The factors were : (1) the five levels (0, 0.1, 0.25, 0.5, and 0.75%); and (2) the ripening time for each parameter. An analysis of variance were performed on all the variables measured using the General Linear Model (GLM) procedure of the SAS statistical package (SAS Inst., 2002). The Duncan's multiple range test ($p<0.05$) was used to determine differences among the treatment means.

## Results and Discussion

**Proximate composition**

Effect of GdL addition on proximate composition of fermented sausages during ripening is given in Table 1.

**Table 1. Effect of GdL addition on proximate composition of fermented sausages during ripening**

| | | Days of ripening | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 | 20 | 25 |
| Moisture (%) | C[1)] | 58.88±0.51$^{Aa}$ | 55.93±0.47$^{Ba}$ | 48.33±0.39$^{Ca}$ | 42.80±0.30$^{Da}$ | 36.31±0.04$^{Ea}$ | 33.93±0.19$^{Ea}$ |
| | T1 | 58.39±0.41$^{Aab}$ | 55.45±0.19$^{Bab}$ | 47.58±0.59$^{Ca}$ | 38.59±0.40$^{Dc}$ | 34.45±0.22$^{Eb}$ | 30.22±0.67$^{Fb}$ |
| | T2 | 58.39±0.66$^{Aab}$ | 55.64±0.40$^{Ba}$ | 48.32±0.06$^{Ca}$ | 39.07±0.45$^{Dc}$ | 33.36±0.50$^{Ec}$ | 30.45±0.42$^{Fb}$ |
| | T3 | 57.93±0.44$^{Abc}$ | 54.66±0.62$^{Bbc}$ | 48.55±0.77$^{Ca}$ | 40.57±0.23$^{Db}$ | 32.52±0.76$^{Ecd}$ | 29.52±0.08$^{Fc}$ |
| | T4 | 57.37±0.29$^{Ac}$ | 54.26±0.44$^{Bc}$ | 44.77±0.73$^{Cb}$ | 37.56±0.38$^{Dd}$ | 32.37±0.47$^{Ed}$ | 29.32±0.16$^{Fc}$ |
| Fat (%) | C | 18.50±0.05$^{Ec}$ | 21.70±0.44$^{Da}$ | 23.47±0.35$^{Cc}$ | 28.87±0.45$^{Ba}$ | 30.61±0.71$^{Ab}$ | 31.16±0.73$^{Ac}$ |
| | T1 | 20.50±0.49$^{Fa}$ | 21.83±0.52$^{Ea}$ | 23.66±0.79$^{Dc}$ | 28.45±0.55$^{Ca}$ | 31.72±0.40$^{Ba}$ | 33.66±0.05$^{Aa}$ |
| | T2 | 19.71±0.19$^{Fb}$ | 21.50±0.59$^{Ea}$ | 24.70±0.45$^{Dab}$ | 28.49±0.79$^{Ca}$ | 31.05±0.35$^{Bab}$ | 33.24±0.33$^{Aa}$ |
| | T3 | 20.10±0.08$^{Fab}$ | 22.14±0.34$^{Ea}$ | 24.18±0.33$^{Dbc}$ | 27.17±0.74$^{Cb}$ | 29.67±0.15$^{Bc}$ | 33.65±0.51$^{Aa}$ |
| | T4 | 18.40±0.51$^{Fc}$ | 19.64±0.22$^{Eb}$ | 25.50±0.45$^{Da}$ | 27.75±0.57$^{Cab}$ | 30.42±0.38$^{Bbc}$ | 32.14±0.25$^{Ab}$ |
| Protein (%) | C | 18.23±0.45$^{Ca}$ | 18.92±0.11$^{Cc}$ | 24.49±0.69$^{Ba}$ | 23.86±0.46$^{Bc}$ | 28.44±0.50$^{A}$ | 28.00±0.59$^{Ac}$ |
| | T1 | 17.73±0.38$^{Dabc}$ | 18.56±0.13$^{Dc}$ | 24.98±0.68$^{Ca}$ | 26.78±0.38$^{Ba}$ | 29.03±0.52$^{A}$ | 29.32±0.69$^{Ab}$ |
| | T2 | 18.05±0.23$^{Fab}$ | 20.28±0.34$^{Ea}$ | 22.27±0.15$^{Db}$ | 25.57±0.44$^{Cb}$ | 28.22±0.39$^{B}$ | 30.96±0.64$^{Aa}$ |
| | T3 | 17.57±0.10$^{Fbc}$ | 19.32±0.28$^{Eb}$ | 21.17±0.21$^{Dc}$ | 25.42±0.35$^{Cb}$ | 28.65±0.38$^{B}$ | 29.48±0.29$^{Ab}$ |
| | T4 | 17.40±0.15$^{Fc}$ | 18.88±0.08$^{Ec}$ | 21.87±0.62$^{Dbc}$ | 26.31±0.16$^{Ca}$ | 28.49±0.57$^{B}$ | 29.67±0.33$^{Ab}$ |
| Ash (%) | C | 3.14±0.02$^{E}$ | 3.56±0.02$^{D}$ | 4.38±0.24$^{C}$ | 4.79±0.16$^{B}$ | 5.14±0.08$^{A}$ | 5.38±0.08$^{A}$ |
| | T1 | 3.12±0.18$^{D}$ | 3.36±0.04$^{D}$ | 4.36±0.25$^{C}$ | 4.75±0.05$^{B}$ | 5.11±0.02$^{A}$ | 5.30±0.02$^{A}$ |
| | T2 | 3.24±0.02$^{E}$ | 3.51±0.03$^{D}$ | 4.29±0.12$^{C}$ | 4.87±0.24$^{B}$ | 5.16±0.03$^{AB}$ | 5.41±0.24$^{A}$ |
| | T3 | 3.21±0.10$^{D}$ | 3.21±0.10$^{D}$ | 3.98±0.07$^{C}$ | 4.71±0.05$^{B}$ | 5.03±0.15$^{A}$ | 5.31±0.25$^{A}$ |
| | T4 | 3.09±0.01$^{D}$ | 3.34±0.07$^{D}$ | 4.06±0.05$^{C}$ | 4.83±0.06$^{Ba}$ | 5.24±0.06$^{A}$ | 5.13±0.28$^{AB}$ |

Each values are reported as means ± standard error of three replicate experiments with three samples analyzed per replicate (n=9).
Means in the same row with different letters (A-F) are significantly different ($p<0.05$).
Means in the same column with different letters (a-d) are significantly different ($p<0.05$).
[1)]C: without GdL; T1: 0.1% GdL; T2: 0.25% GdL;T3: 0.5% GdL;T4: 0.75% GdL.

The differences between the GdL level on proximate composition except the ash contents were significant ($p<0.05$). The moisture contents significantly decreased with increasing GdL concentration and T4 showed the lowest moisture contents ($p<0.05$). These results are in agreement with those reported by some authors (Yilmaz and Zorba, 2010) mentioned the greater the GdL content, the lower the moisture content. This finding is also consistent with Pate *et al*. (1971) who mentioned that moisture contents of hams showed a decrease with increasing amounts of GdL added. The ripening time had effect on the proximate composition of the sausages ($p<0.05$). The moisture contents in all samples significantly decreased throughout ripening from 0 to 25 d ($p<0.05$). On the other hands, the fat, protein and ash contents significantly increased on the 25th d compared to day 0 during ripening ($p<0.05$). A similar trend has been reported by Mendoza *et al*. (2001), who found the reduction in moisture during ripening caused the increase fat contents and protein contents. Previous studies have shown that the fat and ash content increase of sausages during ripening is due to moisture loss by drying (Papadima and Bloukas, 1999).

**Physicochemical characteristics**

Effect of GdL addition on physicochemical characteristics of fermented sausages during ripening is presented in Table 2. The pH values were inversely proportional to the GdL level during ripening. As expected, the higher the added GdL level, the lower pH values ($p<0.05$). This result is confirmed by previous many studies (Hong *et al.,* 2006; Hong *et al.,* 2008; Hong and Chin, 2010; Shin *et al.*, 1991) mentioned the addition of GdL decreased the

**Table 2. Effect of GdL addition on physicochemical traits of fermented sausages during ripening**

| | | Days of ripening | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 3 | 5 | 7 | 10 | 15 | 20 | 25 | SEM[a] |
| pH | C[1)] | $5.69^{Aa}$ | $5.33^{Da}$ | $4.90^{Ga}$ | $5.03^{Fa}$ | $5.40^{Ca}$ | $5.27^{Ea}$ | $5.50^{Ba}$ | $5.41^{Ca}$ | $5.33^{Da}$ | 0.01 |
| | T1 | $5.49^{Ab}$ | $5.32^{Ba}$ | $4.89^{Ha}$ | $5.02^{Ga}$ | $5.30^{Cb}$ | $5.17^{Fb}$ | $5.24^{Eb}$ | $5.27^{Db}$ | $5.32^{Ba}$ | 0.01 |
| | T2 | $5.37^{Ac}$ | $5.30^{Bb}$ | $4.77^{Hb}$ | $4.82^{Gb}$ | $5.00^{Fc}$ | $5.04^{Ec}$ | $5.04^{Ec}$ | $5.16^{Dc}$ | $5.19^{Cb}$ | 0.01 |
| | T3 | $5.14^{Ad}$ | $5.12^{Bc}$ | $4.70^{Hb}$ | $4.73^{Gc}$ | $5.02^{Dc}$ | $4.90^{Fd}$ | $5.03^{Dc}$ | $4.96^{Ed}$ | $5.08^{Cc}$ | 0.01 |
| | T4 | $4.95^{Be}$ | $4.95^{Bd}$ | $4.73^{Fb}$ | $4.65^{Gd}$ | $4.76^{Ed}$ | $4.74^{Fe}$ | $4.80^{Dd}$ | $4.81^{Ce}$ | $5.07^{Ac}$ | 0.01 |
| | SEM | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | |
| WHC | C | $69.22^{Aa}$ | $68.20^{Aa}$ | $55.82^{Da}$ | $54.12^{Da}$ | $60.58^{BCa}$ | $57.30^{Ca}$ | $62.55^{Ba}$ | $59.64^{BCa}$ | $61.07^{BCa}$ | 10.69 |
| | T1 | $68.78^{Aa}$ | $67.83^{Aa}$ | $56.06^{Ca}$ | $53.20^{Ca}$ | $56.54^{Cb}$ | $52.56^{Cb}$ | $59.81^{Ba}$ | $53.87^{Cb}$ | $54.93^{Cb}$ | 11.50 |
| | T2 | $66.23^{Aab}$ | $65.31^{Aab}$ | $51.94^{Cb}$ | $56.55^{Ba}$ | $51.67^{Cc}$ | $55.42^{Bab}$ | $56.47^{Bb}$ | $50.35^{Cbc}$ | $52.64^{Cb}$ | 7.62 |
| | T3 | $63.24^{Ab}$ | $62.31^{Ab}$ | $49.99^{Cc}$ | $49.04^{Cb}$ | $57.76^{Bb}$ | $52.35^{BCb}$ | $53.29^{BCb}$ | $48.39^{Cc}$ | $47.77^{Cc}$ | 10.87 |
| | T4 | $53.41^{Ac}$ | $52.60^{ABc}$ | $48.65^{Bc}$ | $43.84^{Dc}$ | $46.88^{Cd}$ | $49.46^{Bc}$ | $49.52^{Bc}$ | $46.60^{Cc}$ | $43.82^{Dd}$ | 11.24 |
| | SEM | 6.84 | 6.78 | 18.46 | 10.17 | 10.12 | 16.64 | 4.49 | 10.17 | 9.80 | |
| Shear force (kg) | C | $0.30^{Gd}$ | $0.41^{Gd}$ | $1.34^{Fd}$ | $1.61^{EFd}$ | $2.41^{Eb}$ | $3.95^{Dc}$ | $9.82^{Cb}$ | $12.82^{Bb}$ | $13.86^{Ab}$ | 0.26 |
| | T1 | $0.28^{Gd}$ | $0.35^{Gd}$ | $2.05^{Ec}$ | $1.68^{Fd}$ | $2.35^{Eb}$ | $3.88^{Dc}$ | $8.82^{Cb}$ | $12.32^{Bb}$ | $14.17^{Ab}$ | 0.19 |
| | T2 | $0.40^{Fc}$ | $0.67^{Fc}$ | $2.10^{Eb}$ | $2.58^{Eb}$ | $2.22^{Eb}$ | $4.23^{Db}$ | $9.66^{Cb}$ | $12.67^{Bb}$ | $15.95^{Aa}$ | 0.32 |
| | T3 | $0.95^{Fb}$ | $1.26^{Fb}$ | $2.16^{Eb}$ | $2.14^{Ec}$ | $4.05^{Da}$ | $4.39^{Db}$ | $9.52^{Cb}$ | $14.29^{Ba}$ | $16.05^{Aa}$ | 0.13 |
| | T4 | $1.57^{Fa}$ | $1.58^{Fa}$ | $2.69^{Ea}$ | $2.76^{Ea}$ | $4.08^{Da}$ | $4.82^{Da}$ | $12.19^{Ca}$ | $15.84^{Ba}$ | $16.26^{Aa}$ | 0.14 |
| | SEM | 0.01 | 0.01 | 0.02 | 0.06 | 0.11 | 0.02 | 0.27 | 0.64 | 0.73 | |
| VBN | C | $10.99^{H}$ | $16.51^{G}$ | $21.76^{F}$ | $32.56^{E}$ | $35.72^{D}$ | $41.68^{C}$ | $42.32^{C}$ | $51.17^{Bb}$ | $52.80^{A}$ | 0.35 |
| | T1 | $11.73^{H}$ | $15.75^{G}$ | $21.92^{F}$ | $34.59^{E}$ | $35.44^{E}$ | $47.09^{D}$ | $56.29^{B}$ | $55.11^{C}$ | $58.86^{A}$ | 0.25 |
| | T2 | $11.23^{I}$ | $14.83^{H}$ | $20.19^{G}$ | $36.80^{F}$ | $38.05^{E}$ | $44.43^{D}$ | $50.48^{C}$ | $51.66^{B}$ | $60.82^{A}$ | 0.35 |
| | T3 | $11.70^{I}$ | $12.59^{H}$ | $17.62^{G}$ | $24.80^{F}$ | $28.38^{E}$ | $37.63^{D}$ | $47.85^{C}$ | $50.56^{B}$ | $65.02^{A}$ | 0.25 |
| | T4 | $12.20^{G}$ | $11.75^{G}$ | $15.09^{F}$ | $22.51^{E}$ | $26.01^{D}$ | $31.27^{C}$ | $47.64^{B}$ | $51.73^{A}$ | $52.11^{A}$ | 0.22 |
| | SEM | 0.06 | 0.22 | 0.32 | 0.39 | 0.59 | 0.47 | 0.14 | 0.14 | 0.19 | |
| TBA (mg malonaldehyde/kg) | C | $0.83^{E}$ | $0.87^{D}$ | $0.72^{F}$ | $1.11^{A}$ | $0.93^{C}$ | $0.98^{B}$ | $0.94^{C}$ | $0.94^{C}$ | $0.96^{BC}$ | 0.01 |
| | T1 | $0.68^{F}$ | $0.78^{E}$ | $0.83^{CD}$ | $0.96^{A}$ | $0.82^{DE}$ | $0.87^{BC}$ | $0.79^{DE}$ | $0.88^{B}$ | $0.90^{B}$ | 0.01 |
| | T2 | $0.82^{F}$ | $0.85^{F}$ | $0.90^{E}$ | $1.01^{C}$ | $0.95^{D}$ | $1.00^{C}$ | $1.03^{C}$ | $1.09^{B}$ | $1.15^{A}$ | 0.01 |
| | T3 | $0.80^{D}$ | $0.83^{CD}$ | $0.87^{C}$ | $1.02^{A}$ | $0.95^{B}$ | $0.94^{B}$ | $1.01^{A}$ | $0.92^{Bb}$ | $0.95^{B}$ | 0.01 |
| | T4 | $0.65^{F}$ | $0.66^{F}$ | $0.73^{E}$ | $0.90^{D}$ | $1.07^{A}$ | $0.89^{D}$ | $0.89^{D}$ | $0.93^{Cb}$ | $0.96^{B}$ | 0.01 |
| | SEM | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | |

Means in the same row with different letters (A-I) are significantly different ($p<0.05$).
Means in the same column with different letters (a-e) are significantly different ($p<0.05$).
[a]SEM: standard error of the means of three replicate experiments with three samples analyzed per replicate (n=9).
[1)]C: without GdL; T1: 0.1% GdL; T2: 0.25% GdL;T3: 0.5% GdL;T4: 0.75% GdL.

pH of pork. Similar finding was reported by Yilmaz and Zorba (2010), who found that high concentrations of GdL in sausage decrease the pH values. During fermentation, GdL hydrolyzed and transformed to gluconic acid, which was thought to result in a decrease in pH values of the GdL added sausage samples by slow dialysis (Gokalp *et al*., 1997; Ngapo *et al*., 1996). The rate of pH decline was of importance to form an acid-induced protein gel, as a rapid decrease in pH results in severe protein denaturation, causing aggregated protein particulates (Bryant and McClements, 1998). As reported by Chun *et al*. (2014), the pH decreased about 0.4-0.5 units when GdL was added to the formulation. The pH values showed initial rapid decreases during the first 3 d, with subsequent slowly increasing or fluctuating during the ripening period ($p<0.05$). These results are in agreement with those reported by previous authors (Lee *et al*., 2009) noted that the increase in pH during ripening was partially due to the accumulation of the base chemical probably caused by hydrolysis of protein. During the ripening period, the pH values of fermented sausages were lower in sausages with GdL than in samples without GdL ($p<0.05$). As depicted in Table 2, the higher the added GdL level, the lower WHC content ($p<0.05$). The WHC content was the highest ($p<0.05$) in sausages without GdL. Similar results have been reported by previous authors (Chun *et al*., 2014; Hong *et al*., 2006), who indicated that adding GdL decreases the WHC of the restructured pork due to a reduction in pH. The WHC content ranged from 53.41 to 69.22% at day 0 and from 43.82 to 61.07% on the 25th d. The WHC content of sausage samples showed initial decreases during the first 5 d, with subsequent slowly fluctuating or steady. According to the literature review (Briskey, 1964), a high rate of pH decline and low pH result in low WHC in meats. Low WHC could be explained by exhibiting moisture release due to excessive protein denaturation (Barbut, 2010). The shear force values of fermented sausages were higher in T4 (0.75% GdL) than in other treatments ($p<0.05$). Based on previous study (Chun *et al*., 2014), adding GdL increased texture profiles such as hardness and cohesiveness of the pork. Because GdL slowly decreases the pH of pork, it forms an acid-induced meat protein gel (Ngapo *et al*., 1996). During ripening, the shear force values of sausages were significantly increased on the 25th day compared to day 0 ($p<0.05$). As postulated by Mendoza *et al.* (2001), the sausage texture caused an increase in chewiness and hardness at longer ripening times. As indicated in Table 2, the GdL addition had no apparent effect on the VBN and TBA value of the sausages. Juncher *et al*. (2000) investigated that 2.0% lactate+0.25 % GdL improved oxidative stability and led to lower TBARS of meat products. The VBN value of sausages continuously increased with time ($p<0.05$). It remained up to 25 d at values more than 5-60 mg/%. According to the studies of Egan *et al*. (1981), the higher VBN of sausages is explained by accelerated the degradation of protein. The TBARS value of sausage samples showed initial increases during the first 5 d, with subsequent slowly fluctuating or steady. Juncher *et al*. (2000) reported that low pH negatively affected lipid oxidation. TBARS values of samples were higher than the suggested threshold for the appearance of rancidity off flavours in fresh pork (0.5 mg MDA/kg) (Lanari *et al*., 1995). VBN and TBARS values were not influenced by GdL addition in this study.

The influence of GdL addition on color of fermented sausages during ripening is reported in Table 3. CIE L* (lightness) and b* (yellowness) value significantly increased with increasing GdL concentrations, whereas a* (redness) value significantly decreased ($p<0.05$). T4 (0.75% GdL) samples showed the highest CIE L* and b* value when compared to the other treatments ($p<0.05$). This is due to the fact that GdL increases lightness of meat protein gel (Ngapo *et al*., 1996). These results are in agreement with previous studies (Hong *et al*., 2006; Hong *et al*., 2008) noted increasing the GdL level increased the L* value and decreased the a* value of the restructured meat. Kim *et al*. (2010) also investigated that the addition of GdL increased the L* value of cooked rice. Regardless of GdL addition, the L* values in sausages increased slowly during the first 7 d and decreased slowly after 10 d of ripening ($p<0.05$). The a* and b* values of sausages decreased slightly during the ripening period ($p<0.05$). This small decrease in a* values of sausages may be due to the oxidation of nitrosylmyoglobin to nitrate and the brown metmyoglobin (Gøtterup *et al*., 2008). This result was similar to that of the yellowness of Spanish sausage, which decreases during the ripening period (Perez-Alvarez *et al*. 1999). Previous studies have shown CIE L* values did not appear to be influenced by duration of storage, but b* values decreased with storage time (Jeremiah and Gibson, 2001).

**Microbial analysis**

The influence of GdL addition on bacteria count of fermented sausages during ripening is shown in Table 4. Total plate bacteria and *Pseudomonas* counts of sausages were significantly affected by GdL addition ($p<0.05$). The

**Table 3. Effect of GdL addition on meat color of fermented sausages during ripening**

| | | Days of ripening | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 3 | 5 | 7 | 10 | 15 | 20 | 25 | SEM[a] |
| L* | C[1)] | $50.51^{Cc}$ | $50.74^{Cb}$ | $52.32^{Ab}$ | $51.45^{Bd}$ | $52.84^{Ac}$ | $50.26^{Cc}$ | $48.19^{Dc}$ | $47.97^{Dc}$ | $47.32^{Db}$ | 0.03 |
| | T1 | $51.55^{Cb}$ | $51.97^{Ba}$ | $54.42^{Aa}$ | $52.04^{Bc}$ | $53.83^{Ab}$ | $51.45^{Cb}$ | $49.78^{Db}$ | $48.48^{Eb}$ | $47.89^{Fb}$ | 0.08 |
| | T2 | $52.33^{Ba}$ | $52.21^{Ba}$ | $52.86^{Ab}$ | $52.75^{Ac}$ | $53.18^{Ab}$ | $51.28^{Cb}$ | $49.50^{Db}$ | $48.51^{Eb}$ | $47.51^{Fb}$ | 0.11 |
| | T3 | $52.49^{Ca}$ | $51.55^{Da}$ | $54.31^{Aa}$ | $53.75^{Bb}$ | $54.18^{Aa}$ | $52.82^{Ca}$ | $49.09^{Eb}$ | $49.29^{Ea}$ | $47.62^{Fb}$ | 0.04 |
| | T4 | $52.49^{Ba}$ | $52.30^{Ba}$ | $54.67^{Aa}$ | $54.08^{Aa}$ | $54.25^{Aa}$ | $52.69^{Ba}$ | $52.26^{Ba}$ | $49.59^{Ca}$ | $48.25^{Ca}$ | 0.05 |
| | SEM[a] | 0.03 | 0.04 | 0.02 | 0.03 | 0.19 | 0.08 | 0.08 | 0.01 | 0.07 | |
| a* | C | $10.04^{Aa}$ | $5.26^{Fa}$ | $8.13^{Ca}$ | $4.88^{Ga}$ | $8.44^{Ba}$ | $5.93^{Da}$ | $5.59^{Ea}$ | $3.90^{Ha}$ | $3.35^{Ga}$ | 0.01 |
| | T1 | $8.52^{Ac}$ | $4.41^{Cb}$ | $5.61^{Bb}$ | $2.34^{Hd}$ | $5.63^{Bb}$ | $4.17^{Db}$ | $3.97^{Eb}$ | $3.16^{Fb}$ | $2.67^{Gb}$ | 0.01 |
| | T2 | $9.89^{Ab}$ | $2.83^{Ec}$ | $3.45^{Bc}$ | $2.66^{Fc}$ | $3.18^{Cd}$ | $2.93^{Dc}$ | $2.30^{Gc}$ | $2.16^{Hc}$ | $2.18^{Hc}$ | 0.01 |
| | T3 | $8.45^{Ac}$ | $1.53^{Gd}$ | $3.37^{Cd}$ | $2.85^{Db}$ | $3.50^{Bc}$ | $2.15^{Ed}$ | $2.06^{Fd}$ | $1.34^{He}$ | $1.60^{Gd}$ | 0.01 |
| | T4 | $7.32^{Ad}$ | $1.21^{De}$ | $2.78^{Be}$ | $2.72^{Bc}$ | $2.68^{Be}$ | $1.84^{Ce}$ | $1.74^{De}$ | $1.71^{Dd}$ | $1.61^{Dd}$ | 0.01 |
| | SEM[a] | 0.01 | 0.01 | 0.02 | 0.04 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | |
| b* | C | $6.21^{Ab}$ | $4.50^{Be}$ | $4.61^{Be}$ | $4.45^{Bd}$ | $4.29^{Bd}$ | $3.63^{Cb}$ | $2.74^{Dc}$ | $2.68^{Dc}$ | $2.64^{Db}$ | 0.01 |
| | T1 | $6.04^{Ac}$ | $4.84^{Cd}$ | $5.90^{Bb}$ | $4.82^{Cc}$ | $4.83^{Cc}$ | $3.68^{Db}$ | $2.92^{Eb}$ | $2.80^{Eb}$ | $2.57^{Eb}$ | 0.01 |
| | T2 | $6.78^{Aa}$ | $5.68^{Bc}$ | $5.62^{Bc}$ | $4.81^{Cc}$ | $5.66^{Bb}$ | $3.80^{Da}$ | $3.20^{Da}$ | $3.12^{Da}$ | $3.13^{Da}$ | 0.01 |
| | T3 | $6.00^{Ac}$ | $5.80^{Bb}$ | $5.15^{Cd}$ | $5.17^{Cb}$ | $5.79^{Bb}$ | $3.83^{Da}$ | $3.22^{Da}$ | $2.93^{Eb}$ | $3.06^{Da}$ | 0.01 |
| | T4 | $6.63^{Aa}$ | $6.09^{Aa}$ | $6.42^{Aa}$ | $5.30^{Ba}$ | $6.23^{Aa}$ | $3.84^{Ca}$ | $3.28^{Ca}$ | $3.12^{Ca}$ | $3.15^{Ca}$ | 0.01 |
| | SEM[a] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | |

Means in the same row with different letters (A-H) are significantly different ($p$<0.05).
Means in the same column with different letters (a-e) are significantly different ($p$<0.05).
[a]SEM: standard error of the means of three replicate experiments with three samples analyzed per replicate (n=9).
[1)]C: without GdL; T1: 0.1% GdL; T2: 0.25% GdL;T3: 0.5% GdL;T4: 0.75% GdL

**Table 4. Effect of GdL addition on total plate counts and *Pseudomonas* of fermented sausages**

| | | Days of ripening | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 3 | 5 | 7 | 10 | 15 | 20 | 25 | SEM[a] |
| Total plate counts (Log CFU/g) | C[1)] | $6.82^{a}$ | $7.42^{a}$ | $7.61^{a}$ | $7.73^{a}$ | $7.83^{a}$ | $7.67^{a}$ | $7.30^{a}$ | $7.43^{a}$ | $7.18^{a}$ | 0.61 |
| | T1 | $6.82^{a}$ | $7.38^{a}$ | $7.66^{a}$ | $7.71^{a}$ | $7.90^{a}$ | $7.47^{a}$ | $7.28^{a}$ | $7.24^{a}$ | $7.10^{a}$ | 0.52 |
| | T2 | $6.76^{a}$ | $7.15^{a}$ | $7.65^{a}$ | $7.45^{a}$ | $7.54^{a}$ | $7.73^{a}$ | $7.25^{a}$ | $7.03^{a}$ | $7.00^{a}$ | 0.32 |
| | T3 | $6.45^{b}$ | $6.54^{b}$ | $7.19^{b}$ | $7.17^{b}$ | $7.21^{b}$ | $7.23^{b}$ | $7.21^{a}$ | $7.03^{b}$ | $7.14^{a}$ | 0.40 |
| | T4 | $6.36^{b}$ | $6.63^{b}$ | $6.93^{b}$ | $7.01^{b}$ | $7.06^{b}$ | $7.02^{b}$ | $7.01^{b}$ | $7.00^{b}$ | $6.86^{b}$ | 0.51 |
| | SEM[a] | 0.07 | 0.08 | 0.09 | 0.05 | 0.04 | 0.08 | 0.09 | 0.09 | 0.08 | |
| Pseudomonas (Log CFU/g) | C | $6.94^{a}$ | 7.61 | 7.67 | $7.73^{a}$ | $8.07^{a}$ | 7.64 | $7.58^{a}$ | $7.51^{a}$ | 6.90 | 0.54 |
| | T1 | $6.88^{a}$ | 7.66 | 7.53 | $7.76^{a}$ | $7.85^{a}$ | 7.41 | $7.13^{b}$ | $7.09^{a}$ | 6.83 | 0.46 |
| | T2 | $6.75^{a}$ | 7.65 | 7.48 | $7.42^{a}$ | $7.79^{a}$ | 7.68 | $7.04^{b}$ | $7.03^{a}$ | 6.84 | 0.38 |
| | T3 | $6.42^{a}$ | 7.49 | 7.47 | $7.36^{a}$ | $7.49^{a}$ | 7.62 | $7.02^{b}$ | $6.99^{a}$ | 6.77 | 0.59 |
| | T4 | $6.11^{b}$ | 7.23 | 7.27 | $7.16^{b}$ | $7.17^{b}$ | 7.12 | $7.00^{b}$ | $6.80^{b}$ | 6.67 | 0.64 |
| | SEM[a] | 0.05 | 0.12 | 0.10 | 0.06 | 0.07 | 0.17 | 0.03 | 0.09 | 0.13 | |

Means in the same column with different letters (a-b) are significantly different ($p$<0.05).
[a]SEM: standard error of the means of three replicate experiments with three samples analyzed per replicate (n=9).
[1)]C: without GdL; T1: 0.1% GdL; T2: 0.25% GdL; T3: 0.5% GdL; T4: 0.75% GdL.

populations of total aerobic and *Pseudomonas* of T4 (0.75% GdL) samples displayed lower than that of other treatment samples throughout ripening from 0 to 25 d ($p$< 0.05). This could be due to the result in a decrease in pH values of the GdL added sausage samples (Gokalp *et al*., 1997; Ngapo *et al*., 1996), which caused a reduction of bacteria count. Kim *et al*. (2010) investigated that the addition of 1% acetic and GdL was effective in inhibiting the bacteria growth of cooked rice. The count levels did not significantly change throughout all the ripening period ($p$>0.05), even though those showed increases during the 7 d, with subsequent slowly decreasing after 10 d of ripening. The high population of bacteria at day 7 is reflected the addition of the starter culture, which lactic acid bacteria dominated the microflora during fermentation and ripening (Lim *et al*., 2008). Total aerobic counts closely paralleled the *Pseudomonas* bacteria counts (Table 4). The growth of *Pseudomonas* followed closely sensory changes

during storage and thus a growth model for this group could be used for predicting spoilage of stored meat (Koutsoumanis *et al*., 2006). The initial count of total plate bacteria and *Pseudomonas* counts in the sausage mixture was between 6 and 7 Log CFU/g and remained constant until 25 d. This attribute is due to a lack of fermentable carbohydrates (Papadima and Bloukas, 1999). Consequently, we showed that the fermented sausages combined with 0.75% GdL reduced populations of total aerobic bacteria and *Pseudomonas*.

## Conclusions

This result showed that GdL addition significantly affects the proximate composition, physicochemical and microbiological characteristics of fermented sausages during ripening. This indicates that the presence of GdL reduces the pH value of sausages, which can have an antimicrobial effect. Increasing GdL caused a decrease in moisture contents and improved WHC content of the sausage. Adding GdL increased lightness and yellowness of the sausages, whereas VBN and TBA values were not affected by adding GdL. The addition of 0.75% GdL was effective in inhibiting the bacteria growth. GdL could be used as curing agents, as well as nitrite replacement in the production of fermented sausages.

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