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Effect of Glucono-Delta-Lactone on Certain Characteristics of Italian Dry Salami Fermentation under Commercial Manufacturing Conditions.

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There is a constant need for new technologies which will shorten the processing time, increase the uniformity, and assure predictable safety for semi-dry and dry sausages. Introduction of pure lactic acid starters and/or chemical acidulation have assisted the natural fermentation (18,19,27,38).

Use of chemical acidulants such as citric, lactic, sodium acid pyrophosphate, or gluconic acids may accelerate the rate of nitrite reactions which result in the formation of better cured meat color (23). Chemical acidulation may affect meat protein hydration by lowering pH of meat to levels closer to the isoelectric point of the proteins (10). Decrease in protein hydration may increase free moisture and promote dissolution and penetration of cure into the meat substance and accelerate curing. A decrease in protein hydration also increases the rate of drying of the sausage and may shorten processing time (28). Finally, breaking of the sausage emulsion may result from the effect of acidulants on the binding capacity of the meat proteins. Use of glucono-delta-lactone (GDL) in cooked comminuted products such as frankfurters, bologna, and luncheon meats accelerates curing and the development of a satisfactory and stable cured meat color. Glucono-delta-lactone which hydrolyzes slowly to gluconic acid, also does not break the emulsions in emulsion-type sausage since the emulsion is formed before acid formation (23, 24).

GDL has been successfully used in the manufacture of dry and semi-dry sausages. Some advantages claimed for its use are shortening of production time, acceleration of the development of a desired consistency (5,14,20,22), prevention of the activity of microorganisms which cause sausage defects (5) and inhibition of pathogen-like S.aureus and Salmonella (11,16). Sair (23) and Grau (9) claimed that GDL had no effect on normal bacterial growth in sausage. A disadvantage seems to be that addition of GDL may result in a peculiar flavor not characteristic of dry sausage (14,20). Nurmi (21) found that by rapidly decreasing the sausage pH, GDL created favorable conditions for the rapid increase of yeasts and lactobacilli. He also observed (14) that addition of GDL to a dry sausage mix inhibited the naturally occurring micrococci, but not the micrococcal starter culture. Addition of GDL also increased the peroxide number in sausage (5,21). Skulberg (26) found that the addition of GDL considerably accelerated development of the desired consistency, but did not speed up the ripening process of dry sausages and impaired the flavor of the sausages. Kotter et al. (14) found that added GDL decreased the total number of organisms, lactic acid bacteria (LAB), and non-acid forming bacteria. Metaxopoulos et al. (16) demonstrated a measurable growth rate retardation of LAB with increasing levels of GDL from 0 to 1%.

Knowledge of the technology of Italian style dry salami, a fermented sausage produced mainly on the West Coast of the United States, is very limited (4,8,16). This paper discusses the effect of GDL upon selected product characteristics during the fermentation of this salami manufactured under commercial conditions.

MATERIALS AND METHODS:

Sausage formulation and processing: Fifty kilograms of commercial Italian style salami mix prepared as described previously (16), were taken from a commercial batch and divided into five equal parts. The basic salami ingredients included: meat 89.32% (fresh bulk meat 15.7%, frozen pork 84.3%), dry skim milk 3.5%, sugar (dextrose) 3.1%, NaCl 3.2%, spices (black and white pepper, nutmeg, cinnamon, dry garlic, wine) 0.836%, $NaNO_2$ 0.014%, $NaNO_3$ 0.010%, and natural starter (fermenting salami) 0.017%. Natural lactic starter in the form of 18-19 day old, aging sausage batch to assure a level of at least 1×10^5/g LAB was also added to the salami mix. Amounts of 0%, 0.25%, 0.50%, 0.75%, or 1% GDL powder (Griffith Laboratories) were added to each of five salami mix portions and the mixes were ground twice (10 mm plate) for even distribution of GDL. A manually operated stuffer was used to stuff the mix into fibrous casings of 6 cm diameter (Union Carbide) to make standard 13 oz (396 g) salamis. Strings of 4 sausages/string were fermented at 16°C and 88-90% relative humidity (RH) for 24 hours and then at 24-26°C and 75-80% RH for 2 days. Mold growth and ripening was completed at 15-18°C and 75-80% RH for 18 additional days.

Individual salamis were taken from each treatment immediately after stuffing and at 1,2,3,7,14, and 21 days, placed in plastic bags inside cardboard boxes, and shipped by bus to the University laboratory (a 2 hour trip) in the late afternoon. The salamis were refrigerated overnight and analyzed the next morning for microbiological, chemical, and rheological characteristics.

Microbiological assays: The salamis were sliced into two approximately equal parts and samples were taken from cross sections on either side of the center of the sausage. Samples (25 g) were mixed with 225 ml of sterile water and blended for 3 minutes. Appropriate dilutions were plated. Total aerobic counts were determined on APT (Difco) agar incubated at 25-28°C for 2 days. Total lactic acid bacteria (LAB) were determined on Rogosa SL agar (Difco) incubated in jars with candles at 25-28°C for 3-4 days. Salt tolerant cocci were determined on mannitol salt agar (Difco) incubated for 2 days at 37°C and yeasts on potato dextrose agar (Difco) incubated for 3 days at 25-28°C. Microbial colonies on agar media were tested for catalase production and Gram staining. Catalase negative colonies on APT and Rogosa SL agar were counted as LAB.

Chemical assays: Moisture, nitrite, pH and NaCl were measured according to AOAC methods (3). Brine % was derived from the formula: brine % = [NaCl%/(NaCl% + water%)] x 100. Titratable acidity was determined by titrating the 250 ml slurry used for microbiological analysis to pH 7.0 with 1 N NaOH. From the amount of NaOH used, the percent total acid was calculated as lactic acid. Water activity (a_w) was measured with a Sina hygroscope (Model SJT-B Sina-Beckman) after an equilibration period of 1 hour at 25°C. Only the core of the salami (5 cm diameter) was used. Development of cured meat pigment (color) as nitric oxide myoglobin was

measured according to Hornsey (12) using 5 g salami samples. The extract release volume (ERV) measurement was done as described by Acton et al. (2) using duplicate 25 g salami samples, 100 ml distilled water and 30 minute filtration at 25°C. Shear forces for slices of salami were measured with a L.E.E. Kramer Shear Press. Slices 2.5 cm thick and 4 cm in diameter were prepared from the core of each salami. The shear force was calculated as Kg force/g sample/cm^2 surface area exposed to shear blades.

RESULTS AND DISCUSSION:

With the exception of the data presented in Fig. 1, the rest of the reported data represent mean values of six samples for each treatment. The relationship between the amount of GDL added to salami mix and the pH observed after an overnight refrigeration (based on 2 experiments) is shown in Fig. 1.

The effect of GDL on the pH of the salami during fermentation and drying is shown in Fig. 2. After 21 days of processing there was no difference in the pH (approximately 4.7) of salamis with or without 0.25% GDL. An increasingly lower final pH was obtained with increasing levels of GDL. Increasing levels of added GDL resulted in a faster rate of decrease and lower levels of pH during the first 24 hours. In all salamis, the pH decreased rapidly during the first 3 days of fermentation, and more slowly between the 3rd and the 7th day. There was very little pH decline between the 7th to 21st day of processing. Decrease of pH in all salamis resulted from lactic acid production by the LAB flora and the hydrolysis of GDL. A decrease of pH after the leveling of LAB growth may have been due to the increasing concentration of acid because of dehydration. Titratable acidity was 1.58% in salami with the GDL and 1.4% in the salami with no GDL. This small difference probably reflects a suppression of lactic acid production capability of the LAB by GDL at the 1% level. No analysis for gluconic acid was done.

As expected, the rate of residual nitrite disappearance was greatly affected by the amount of GDL added and by lowering of pH (6,23,28). The higher the % GDL, the faster the decline of nitrite during the first 3 days of fermentation. At all levels of GDL, a minimum of about 10 ppm nitrite was observed by the 7th day. Nitrite increased to 12-20 ppm by the 14th day, then declined to 0-9 ppm by the 21st day. Although higher levels of residual nitrite were observed on the 14th day with increased levels of GDL, nitrite level in fully ripened salami was not related to amount of GDL used. Skjelkvale et al. (25) reported similar findings for Norwegian dry sausage. We assume that increased nitrite was due to reduction of residual nitrate, but whether this reduction is of chemical or microbiological origin was not determined. Increased GDL inhibited the growth of nitrate-reducing cocci in the salami.

Development of cured meat pigment (color) appeared as residual nitrite started disappearing. Increasing levels of GDL resulted in faster development and higher levels of nitric oxide myoglobin (Fig. 2). In salami made with 0.75 or 1% GDL, more cured meat pigment was developed within 24 hours than in any of the control salamis at any time of processing. The reason for a small and temporary deterioration in cured meat pigment on the 2nd day of fermentation of the salamis with 0.75 and 1% GDL is not clear. It may be related to decreased extractability of nitric oxide myoglobin at a pH close to its isoelectric point.

In 21 day finished salamis, a_w's of 0.864 and 0.886 were recorded in the presence of 1 or 0% GDL respectively. Skjelkvale et al. (25) observed that addition of nitrate, GDL, or starter culture did not influence the a_w in Norwegian dry sausage. Slightly higher brine concentration was observed in fully ripened salami with GDL than without GDL. This is similar to previous findings (16). A lower moisture content was observed in finished salamis with GDL than controls, which is in agreement with Kotter et al. (14). Skjelkvale et al. (25) found no difference in weight loss of Norwegian salami sausage with or without 0.5% GDL, while Nurmi (20) reported 1-2% more weight loss in the presence of 1% GDL compared to no GDL. In general, sausages with high initial pH are expected to lose the least moisture because of increased protein hydration (10,17,28). Different results among investigators may reflect compositional variations in the salamis and microbial processing differences.

The ERV was used as an indicator of change in the meat water-holding capacity (WHC) during the stages of fermentation and drying (Fig. 3). In salamis without GDL, the maximum ERV (minimal WHC) value appeared about the 7th day of processing when the pH of the salami was approximately 4.75. For the 0.25,0.5,0.75, and 1% GDL levels, the maximum ERV was observed at days 14,14,1, and 0 respectively. This probably reflects the time the pH of the sausage approached 5.1, approximately the I.P. of actomyosin (10). The data of Fig. 3 indicate that the ability of the salami to bind water is not dependent only upon the pH, but also upon the native state of meat proteins, the amounts of GDL, and biochemical changes caused by microbial activity.

Shear force values increased with increased levels of GDL and time, as shown in Fig. 4. Within 24 hours after addition of 0.25% GDL, the consistency of salami which could be sliced easily, was denser than 21 day old salami without GDL. The increased shear force was due not only to the gradual dehydration of the product (1,13), but also to the direct effect of GDL upon the hydration state of meat proteins. Hydration state was mediated by the rate of pH decease in the salami mix. Released water can be easily absorbed by glucose and dry milk solids which are added to the mix in substantial amounts. Similar data have been reported by others (5,14,15,20,25).

Development of a peculiar flavor in salamis with GDL reported by others (14,20) was also observed by a panel of three in the present study when the level of GDL was higher than 0.25%.

Fig. 5 indicates the effect of GDL on selected microbiological changes during fermentation. Increasing levels of GDL extended the lag phase, decreased the maximum level of growth and resulted in fewer LAB in the finished product. The effect of 0.25% GDL on these bacteria was small, but noticable. Our results agree with those of Kotter et al. (14) and Hopke and Schlagel (11), but disagree with those reported by others (9,20,23), who indicated that added GDL either had no effect on normal lactic flora of sausage or favored its growth. These differences may be the result of variations in sausage formulation, processing parameters, and the natural flora of the sausages studied. The effect of GDL upon the total numbers of aerobic organisms in salamis was similar to that observed for the LAB. Addition of

GDL was especially inhibitory to the growth of salt tolerant cocci. Some of the salt tolerant cocci in salami with GDL died and most had an extended lag phase. This is in agreement with Nurmi (20). A slight inhibitory effect of GDL on yeast growth, especially at the 0.75 and 1% levels, was also observed. After 21 days drying, there were 10 times fewer yeasts in salamis with 1% GDL than in salamis without GDL. This contradicts Nurmi's claims (21) that rapid decrease of sausage pH with GDL favors the rapid growth of yeasts. Microscopic examination of colonies grown on APT and Rogosa agars indicated the rapid disappearance of Gram negative bacteria and Gram positive catalase negative rods by the 3rd day of fermentation, especially in salamis with increased levels of GDL. Other than decreasing rate of growth with increasing levels of GDL, the types of LAB did not differ in salamis with or without this acidulant.

Our findings indicated that the cured meat pigment and consistency of salamis made with natural starter culture was improved by the addition of not more than 0.25% GDL. The processing time of this salami may have been shortened slightly. The beneficial effect of GDL on the safety of Italian dry salami with regard to Staphylococcus aureus and Salmonella has been reported (11,16). Contrary to the findings of others, the addition of GDL at levels greater than 0.25% did have an impact on the growth of LAB in the products studied. Such levels of GDL may also result in a sweet, undesirable flavor.

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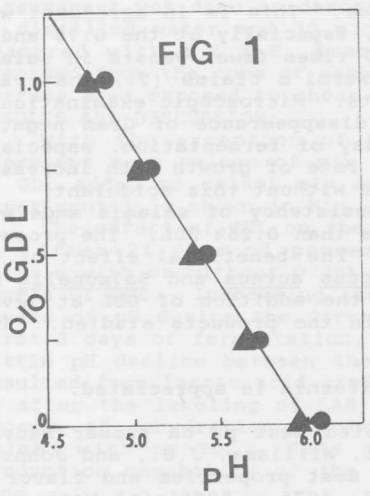
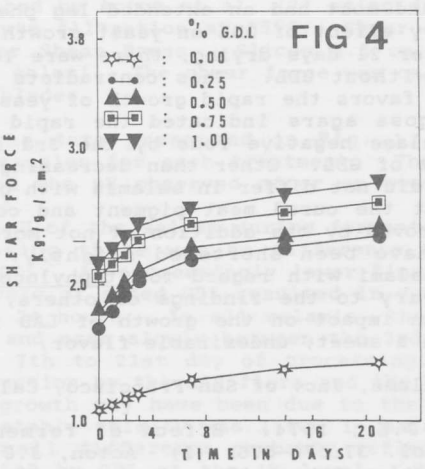
REFERENCES:

(1) Acton, J.C. and Keller, J.E., 1974. Effect of fermented meat pH on summer sausage properties. J. Milk Food Technol. 37:570-576. (2) Acton, J.C., Williams, J.G., and Johnson, M.G., 1972. Effect of fermentation temperature on changes in meat properties and flavor of summer sausage. J. Milk Food Technol. 35:264-268. (3) AOAC, 1975. "Official Methods of Analysis," 12th Ed. Association of Official Analytical Chemists, Washington, D.C. (4) Cahalan, D.L., and Genigeorgis, C., 1986. Study of the microbial flora during the natural commercial fermentation of Italian dry salami and identification of species suitable as starters. Proc. 32nd European Meet. of Meat Res. Workers. (5) Coretti, K., 1966. Schnellreifung von Rohwursten, Glucono-delta-Lacton, ein nitrisparender Hilfsstoff. Fleischerei. 17:5. (6) Fox, J.B.,Jr., 1966. The chemistry of meat pigments. J. Agri. Food Chem. 14:207-210. (7) Genigeorgis, C. 1976. Quality control for fermented meats. J.A.V.A. 168:1220-1228. (8) Genigeorgis, C., Wilson, B., Fanelli, M.J., and Metaxopoulos, J. 1986. Effect of processing parameters on certain microbiological and biochemical characteristics of fermented Italian-style dry salamis manufactured under commercial conditions. Proc. 32nd European Meet. of Meat Res. Workers. (9) Grau, R., 1965. Glucono-delta-Lacton und seine Anwend-barkeit bei der Herstellung von Fleischerzeugnissen. Fleischwirtschaft. 45:1455-1456. (10) Hamm, R., 1960. Biochemistry of meat hydration. In C.O. Chichester, E.M. Mrak, and G.F. Stewart (ed). Advances in Food Research. Academic Press, New York. pp. 428-433. (11) Hopke, H.O. and Schlagel, E. 1980. Studies on the influence of an addition of glucono-delta--lactone to raw sausage meat on the inactivation of a Salmonella species. Proc. World Cong. Foodborne Infections and Intoxications. pp. 877-879. (12) Horsney, H.C., 1956. The colour of cooked cured pork. I Estimation of nitric oxide-haem pigments. J. Sci. Food Agr. 7:534-540. (13) Keller, J.E., Skelley, G.C. and Acton, J.C., 1974. Effect of meat particle size and casing diameter on summer sausage properties during drying. J. Milk Food Technol. 37:101-105. (14) Kotter, L., Palitzsch, A. and Kundrat, W., 1969. Zur Bedeutung von Glucono-delta-Lactone Beider Reifung von Rohwurst. Fleischwirtschaft. 49:321-326. (15) Meester, J. 1965. The application of glucono-delta-lactone in meat products. XI European Meet. Meat Res. Workers, Beograd. p.7. (16) Metaxopoulos, J., Genigeorgis, C., Fanelli, M.J. et al. 1981. Production of Italian dry salami: Effect of starter culture and chemical acidulation on staphylococcal growth in salami under commercial manufacturing conditions. Appl. Environ. Microbiol. 42:863--871. (17) Niinivaara, F.P., 1954. Die Bedeutung des Kochsalzes fur die Herstellung von Roh und Bruhwurst. Fleischwirtschaft. 6:356, (18) Niinivaara, F.P., 1955. Uber den Einfluss von Bakterienrein Kulturen auf die Reifung und Umrotung der Rohwurst. Acta Agr. Fenn. 84:1-128. (19) Niven, C.F., Jr., Deibel, R.H., and Wilson, G.D., 1958. The AMIF sausage starter culture. Amer. Meat Inst. Found. Cir. no 41. (20) Nurmi, E., 1966a. Studies on the acceleration of the ripening process of dry sausage. XII Eur. Meet. of Meat Res. Workers, Sandfjord. p. 21. (21) Nurmi, E., 1966b. Effect of bacterial innoculations on characteristics and microbial flora of dry sausage. Acta. Agric. Fenn. 108:1-77. (22) Nurmi, E., 1968. Effect of pH value on the development of consistency of dry sausage. XIV Eur. Meet. of Meat Res. Workers, Brno. p. 1. (23) Sair, L., 1963. A,B, and C of meat curing. Nat. Provis. 148:18-21. (24) Sair, L. and Henry, W.E. 1967. Glucono-delta-lactone and sausage manufacture. Proc. 10th Annual Conference of the Canadian Institute of Food Technologists, Montreal, Canada. (25) Skjelkvale, R., Tjaberg, T.B., and Valland, M., 1974. Comparison of salami sausage produced with and without addition of sodium nitrite and sodium nitrate. J. Food Sci. 39:520-524. (26) Skulberg, A., 1966. Cited by Nurmi, 1966b. (27) Stoychev, M., Dejejeva, G., and Niinivaara, F.P., 1972. On the biodynamics of starter cultures. XVIII Eur. Meet. of Meat Res. Workers, Guelph. p. 82. (28) Townsend, W.E., Davis, C.E., and Mercuri, A.J., 1975. Effect of chemically adjusted meat pH and drying air velocity on some properties of dry sausage. J. Milk Food Technol. 38:764-768.





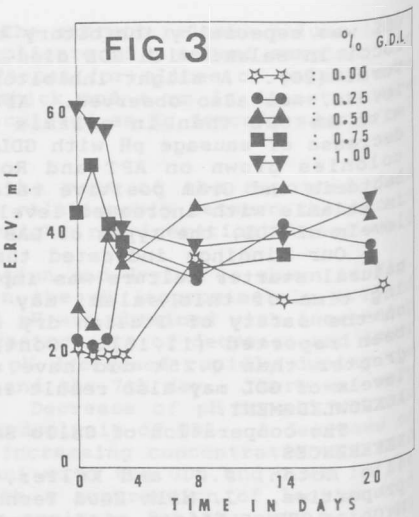


Figure 1. Relationship between percent GDL added to commercial salami formulation and pH after overnight refrigeration (based on two experiments).

Figure 3. Effect of GDL added to salami upon extract release volume changes observed during commercial manufacturing.

Figure 4. Effect of GDL added to salami upon its texture during the commercial manufacturing process.

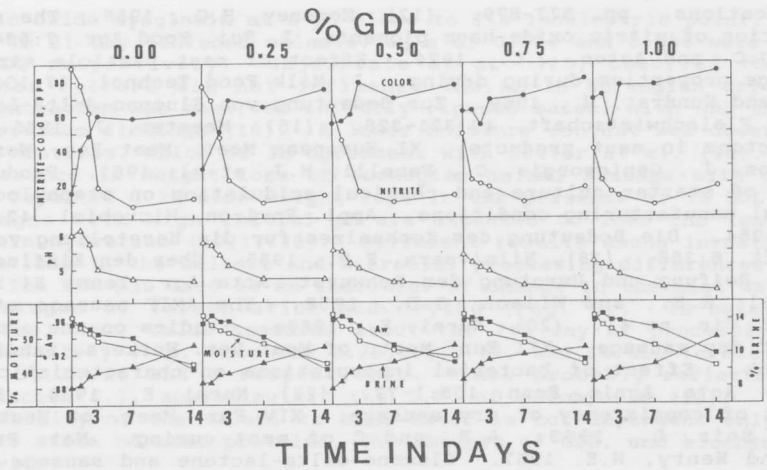


Figure 2. Effect of GDL added to salami formulation upon the pH, residual nitrite, cured pigment (color), a_w, % brine and moisture levels observed during commercial manufacturing.

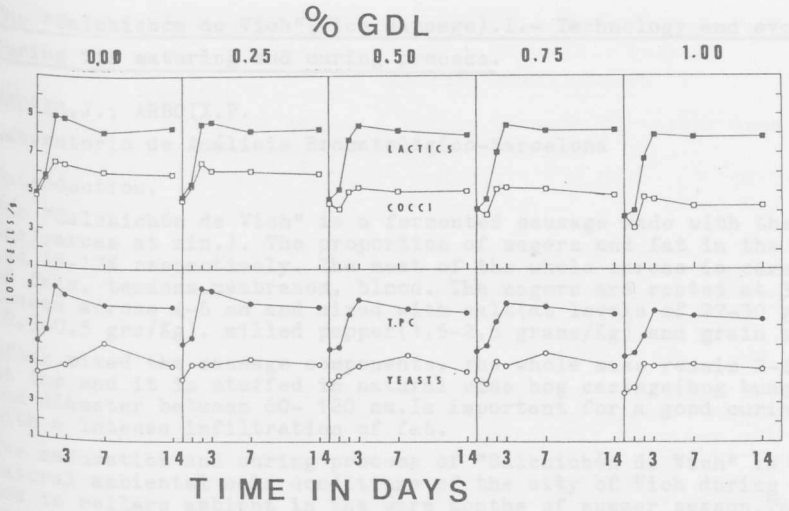


Figure 5. Effect of GDL upon the growth of lactobacilli (lactics), salt tolerant cocci, total aerobic microorganisms and yeasts during commercial salami manufacturing.