facts

Microbiological safety of raw-fermented sausages with Glucono-delta-Lactone

Jungbunzlauer

From nature to ingredients®

Introduction

Salami is a raw-fermented, cured sausage that historically originates in Europe where it has been consumed for centuries. Nowadays you can find salami in almost every region of the world and each country or region has its own mixture of meat, salt and seasoning and its own fermentation technique. The different raw materials and processes result in a vast range of raw-fermented sausages around the world.[1]

Salami was formerly produced using two techniques: drying of the meat and spontaneous fermentation with the indigenous meat flora. However, these processes were poorly understood, resulting in often faulty end products. Nowadays, the majority of raw-fermented sausages are produced with the help of starter cultures, which usually contain at least one strain of lactic acid bacteria. The lactic acid helps to coagulate the proteins through a decrease in pH, thus creating a hostile environment for other undesirable microorganisms. In addition, the starter culture itself is an obstacle to pathogens because they contain competitive flora.[1]

Glucono-delta-Lactone (GdL) can be used instead of starter cultures in the production of raw sausages. After the addition of GdL, pH decreases immediately. The decrease in pH depends on the dosage of GdL and therefore a desired target pH can be reached precisely.

Challenges in the production of raw-fermented sausages

On the European Commission's portal RASFF – Rapid Alert System for Food and Feed – it can be seen that repeatedly raw-fermented sausages are contaminated mainly by *Salmonella* but also by *Listeria monocytogenes*.[2] Initial high amount of pathogens, caused by insufficient quality of meat or of processing methods, can survive due to the lag phase of the starter cultures. The lag phase is the first stage (up to 17 h) of fermentation during which the starter cultures have to adapt to the environment and need to grow. They do not produce lactic acid during this phase. Therefore, the lag phase offers an opportunity for pathogens in the sausage to multiply. Since GdL provides acidification immediately after its addition (within 1 h), making it more of a challenge for pathogens to grow, it can help to bypass the critical lag phase.

In a challenge study the behaviour of *Listeria monocytogenes* and *Salmonella enterica* during a semi-dry salami (25% water loss) manufacturing process was evaluated. *Listeria* is an ubiquitous pathogen which frequently occurs in raw pork but can also be found on processing equipment. It has the ability to survive at low temperatures and even grows during storage in the fridge.[3] *Salmonella* is a typical contaminant of raw meat and was found by Martin et al. (2011) in 23.7% of all raw material samples taken from 10 Spanish sausage producers.[4]

The evaluation was performed on three different salami batches produced with Glucono-delta-Lactone alone, Glucono-delta-Lactone plus a standard starter culture and a starter culture alone. The purpose of the challenge test was to confirm the hypothesis that the combination of both acidification techniques leads to increased microbiological food safety.

Methodology

The study was conducted at IRTA Institute for Food and Agriculture Research and Technology in Monells, Spain. Three batches of salami were manufactured following a typical procedure consisting of preparation of the meat batter, stuffing, fermentation, drying and subsequent storage. Batch 1 contained GdL F5010 from Jungbunzlauer but no starter culture, batch 2 contained GdL F5010 and a starter culture of *Lactobacillus sakei* and *Staphylococcus carnosus*, while batch 3 contained the aforementioned starter culture but no GdL.
Lactobacillus sakei belongs to the lactic acid bacteria (LAB), while *Staphylococcus carnosus* belongs to the Gram-positive catalase positive cocci (GCC+).

Recipe ingredients

Batch 1: 40% pork trimmings (-6°C), 35% lean pork shoulder (-6°C), 25% back fat (-20°C), 28 g/kg nitrite salt (with 0.5% NaNO2), 1.0 g/kg white pepper, 2.0 g/kg black pepper, 0.5 g/kg sodium ascorbate, 2.0 g/kg dextrose, 8.0 g/kg GdL F5010

Batch 2: 40% pork trimmings (-6°C), 35% lean pork shoulder (-6°C), 25% back fat (-20°C), 28 g/kg nitrite salt (with 0.5% NaNO2), 1.0 g/kg white pepper, 2.0 g/kg black pepper, 0.5 g/kg sodium ascorbate, 2.0 g/kg dextrose, 8.0 g/kg GdL F5010, 25 g/50 kg starter culture

Batch 3: 40% pork trimmings (-6°C), 35% lean pork shoulder (-6°C), 25% back fat (-20°C), 28 g/kg nitrite salt (with 0.5% NaNO2), 1.0 g/kg white pepper, 2.0 g/kg black pepper, 0.5 g/kg sodium ascorbate, 2.0 g/kg dextrose, 25 g/50 kg starter culture

In batches 1 and 2, GdL was added to the meat batter just before the addition of pathogens.

Inoculation of pathogens

Batches 1, 2 and 3 were independently inoculated with a cocktail of three strains of *L. monocytogenes* (CTC1034, 12MOB045LM, Scott A) and three strains of *S. enterica* (CTC1003, CTC1022, CTC1754) mixed with water, to reach ca. $1x10^5$ cfu/g in the meat batter per species.

Stuffing

Fibrous casings with a 58 mm diameter were previously dipped into a pimaricin solution in order to prevent mould growth during ripening. The casings were then vacuum-filled with the inoculated meat batter.

Processing conditions for fermentation, ripening and storage

Fermentation and ripening took place in a Versatile Environmental Test Chamber MLR-350H (Sanyo Electric Co., Ltd. Japan) adapted with a Hygrotest 600 PHT -20/120 transmitter (Testo). The fermentation and ripening process followed the scheme described in table 1.

Table 1: Salami manufacturing conditions using GdL, GdL + starter cultures, starter cultures

Time (h)	Temperature (°C)	Relative Humidity (%)
3	25	85
21	24	94
24	22	92
24	20	90
24	16	86
X*	16	84

* Salami ripening was stopped after a weight loss of 25% was achieved. The salamis were subsequently vacuum-packed and stored at 7°C for 6 days.

Analytical determinations

Physico-chemical determinations (pH, water activity (a_w), weight loss and microbiological counts (Gram +catalase +cocci, *Listeria*, *Salmonella*)) were performed in triplicate on day 1, day 4 and day 7, after achieving a 25% weight loss and after 6 days of storage at 7°C.

Results from the physico-chemical and microbiological evaluation were compared through analysis of variance (ANOVA). Significance level was set at $\alpha<0.05$.

Results

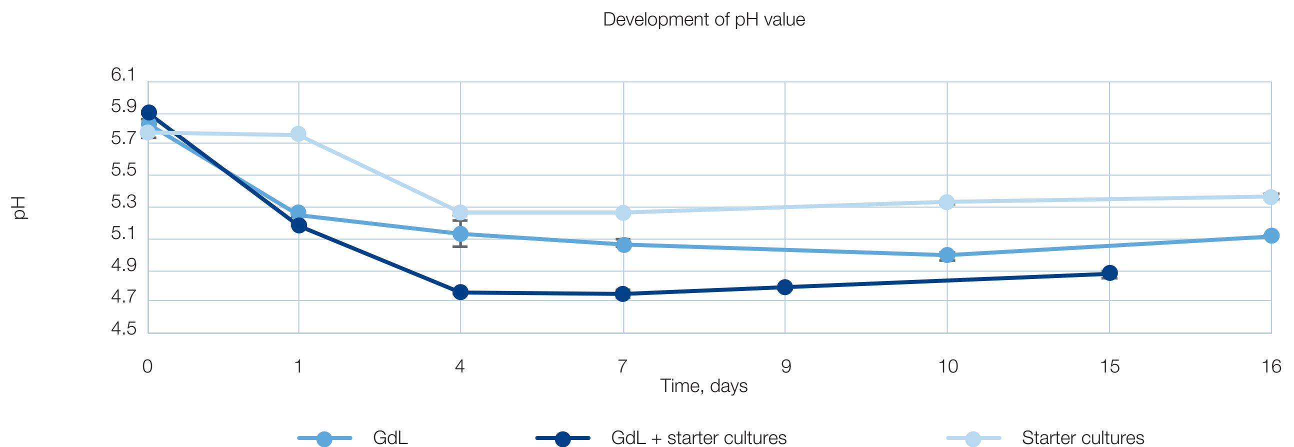
Physico-chemical analysis and technological microbiota

The desired 25% weight loss was first achieved within 9 days by the combination of GdL and starter cultures (batch 2), while batches 1 and 3 required 10 days. This results in a total processing time of 9 and 10 days, respectively. After fermentation and drying, the salamis were stored for 6 days as described above. Accordingly, the total study lasted 15 days (batch 2) and 16 days (batches 1 and 3), respectively.

The water activity of all batches was similar at day 0, being 0.97. As a result of drying, the a_w-value decreased to 0.95, with no significant differences between the three batches.

Figure 1 shows the pH of the three batches throughout the study. The pH in batch 1 and batch 2 (containing GdL) decreased immediately, whereas batch 3 (with starter cultures alone) showed no significant decrease in pH over the first day. At the end of the study the combination of GdL and starter cultures led to the greatest decrease in pH (4.87), followed by GdL alone (5.12) and starter cultures alone (5.37).

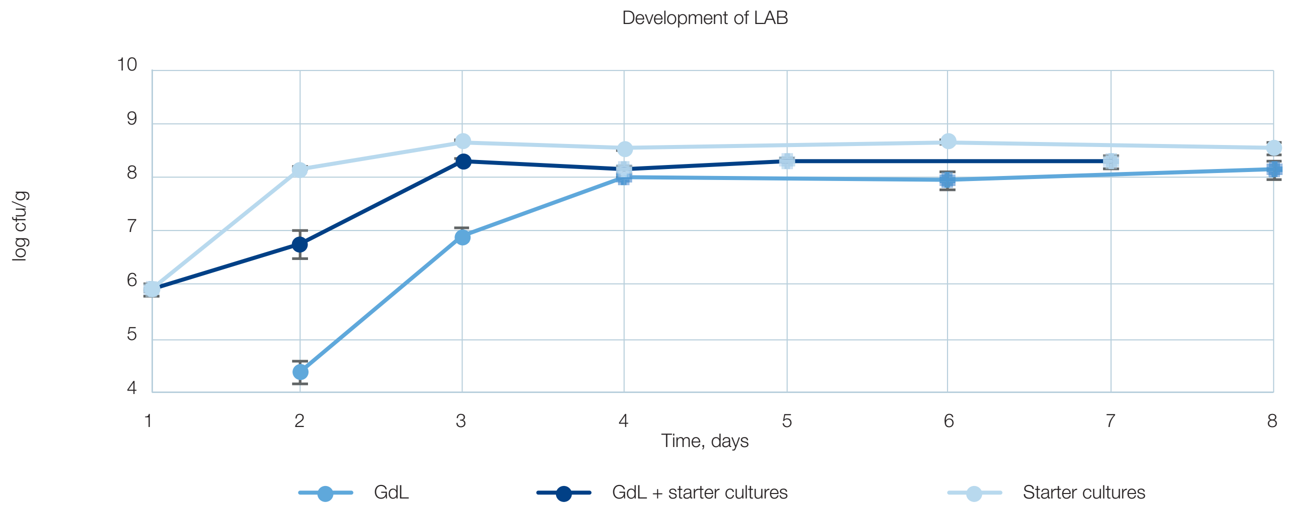
Figure 1: Development of pH in salamis with GdL, starter cultures and a combination of both



As expected, the bacterial counts of lactic acid bacteria (LAB) and Gram-positive catalase positive cocci (GCC+) were already high on day 0 due to the addition of starter cultures to batches 2 and 3 (approx. 6 log cfu/g). They increased further throughout the study.

In batch 1 produced with GdL only, no LAB or GCC+ were added. However, each meat matrix may contain an endogenous mixed flora. Therefore, LAB and GCC+ growth occurred here as well. The endogenous LAB in this batch were below the detection limit in the meat batter on day 0. They increased throughout processing, fermentation and storage, reaching 8 log cfu/g on day 16. This value was significantly lower than the counts in the batch with starter cultures only (8.53 log cfu/g) but not significantly lower than the counts in the batch with the combination of both (8.29 log cfu/g). GCC+ developed analogously to the LAB growth. Figure 2 shows the growth of lactic acid bacteria in the three batches during the study.

Figure 2: Growth of lactic acid bacteria in salamis with GdL, starter cultures and a combination of both

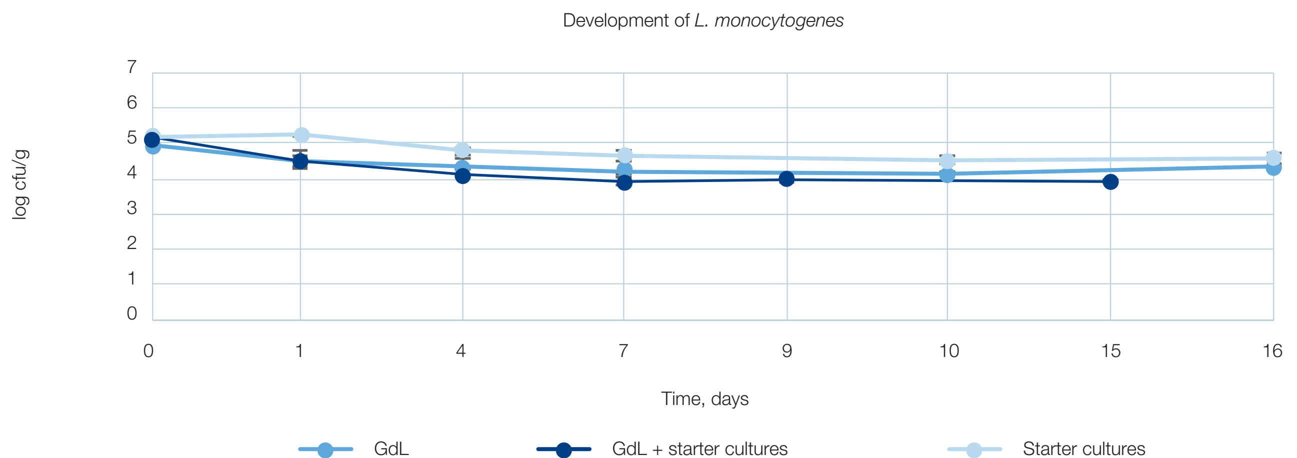


Pathogenic bacteria

L. monocytogenes and *S. enterica* are able to grow at a_w-values of over 0.92. Survival of *L. monocytogenes* was reported at pH values as low as 4.0[5] whereas *S. enterica* can survive at pH 4.4[3]. That makes the salami product presented in this study microbiologically unstable and favourable to the survival of the pathogens mentioned. Neither *L. monocytogenes* nor *S. enterica* grew under the described formulation and processing conditions. Nevertheless, the development of both pathogens was significantly different in all batches. The development of *L. monocytogenes* and *S. enterica* is shown in figures 3 and 4 respectively, from processing until end of storage at day 16.

Within the first day, *L. monocytogenes* counts had already decreased in batch 1 (0.42 log reduction) and in batch 2 (0.68 log reduction), whereas the batch treated with starter cultures alone did not show a decrease in *L. monocytogenes*. By the time the targeted 25% weight loss was reached, *L. monocytogenes* had decreased by 1.21 log in the batch with the combination of GdL and starter cultures, 0.81 log in the batch with GdL alone and 0.66 log in the batch with starter cultures alone. At the end of the storage time (15 or 16 days), a total reduction of 1.23 log (GdL and starter cultures), 0.64 log (GdL) and 0.61 (starter cultures) was observed.

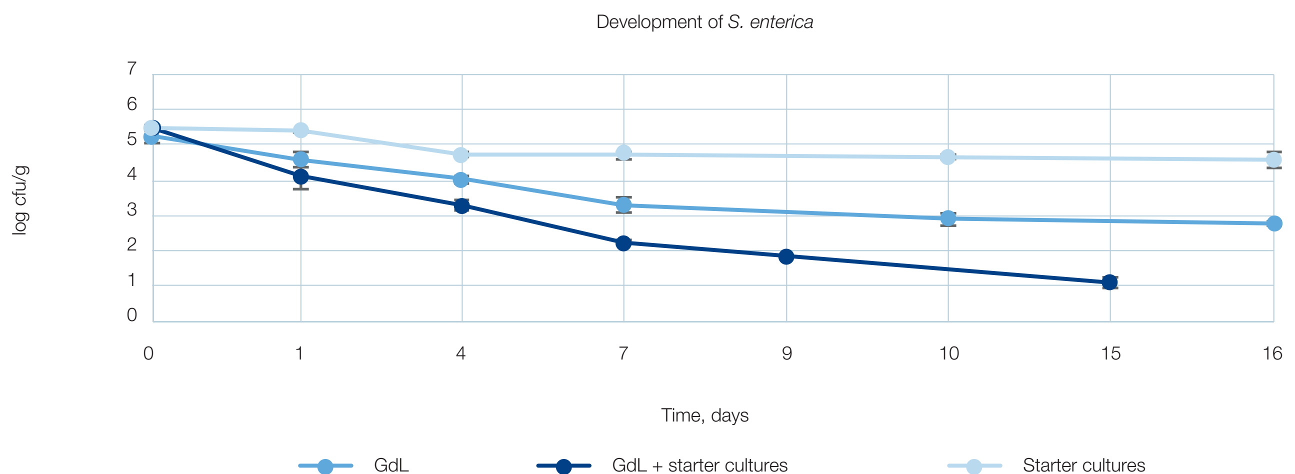
Figure 3: Development of *L. monocytogenes* counts



The counts of *S. enterica* were considerably more affected by the addition of GdL. Again, the reduction of *Salmonella* in the batches containing GdL was more pronounced than in the batch with starter cultures only (see figure 4). Within the first day, *Salmonella* had decreased significantly by 1.34 log in the batch with GdL and starter cultures (batch 2) and by 0.69 log in the batch with GdL alone (batch 1), while the reduction in the batch with starter cultures alone (batch 3) was not significant (0.11 log). By the time the targeted weight loss was reached (day 9 resp. 10), *S. enterica* counts had decreased by 3.63 log (GdL and starter cultures), 2.38 log (GdL) and 0.82 log (starter cultures).

The batch with GdL and starter cultures was the only one that showed a significant further reduction of *Salmonella* during storage, leading to a total reduction of 4.40 log during the entire study. The *Salmonella* counts in batches with GdL and starter cultures alone did not decrease significantly during storage, with total reduction of 2.51 log (GdL) and 0.91 log (starter cultures).

Figure 4: Development of *S. enterica* counts



There is a clear correlation between decrease in pH and inactivation of pathogens, raising the question of whether a lower pH value in the salami with starter cultures would have led to a similar pathogen reduction. However, pH in the batch with GdL and starter cultures did not decrease further from day 4 onwards, whereas the *Salmonella* counts did continue to fall. The same effect, although less pronounced, can be seen in the batch with GdL alone, but not in the batch with starter cultures alone. This observation indicates that GdL might have a positive effect on the reduction of *Salmonella* beyond its pH-lowering effect.

It should be emphasised that this study demonstrates a single scenario and various parameters could be modified to explore further the advantages offered by the combination of GdL and starter cultures.

Summary

This paper demonstrates the innovative idea of combining Glucono-delta-Lactone and starter cultures in raw-fermented sausages in order to reduce *Salmonella* and *Listeria* counts and to achieve a microbiologically safer food product.
Both pathogens were affected by the addition of Glucono-delta-Lactone, resulting in reductions up to 4.4 log for *S. enterica* and 1.23 log for *L. monocytogenes*. The combination of GdL and starter cultures led to a rapid and greater decrease in pH, which provoked a significant and greater reduction of *L. monocytogenes* and *S. enterica* than starter cultures alone. Particular emphasis should be placed on the fact that the decrease in *Salmonella* counts continued in the batch with GdL and starter cultures, independent to changes in pH.

Considering that the inoculation level in this study was noticeably higher than the typical pathogen levels of about $1x10^2$ cfu/g in contaminated meat,[6] it can be assumed that GdL provides even greater safety in naturally contaminated products. When the initial pathogen level is lower, GdL may potentially help to produce a final product free of pathogens. It is worth noting that these results have been achieved in salami which has a relatively high water activity of 0.95 and has not been smoked, and therefore favours the growth of pathogens such as *Listeria* and *Salmonella*.

EU food legislation stipulates no minimum requirements for pathogen reduction in sausage fermentation and ripening. Hence producers are required to base their processes on the hazard analysis and critical control point (HACCP) system and to demonstrate the safety of their production practices. The addition of ingredients such as GdL to reduce potential pathogens enables manufacturers to improve their processes and reduces the risk of microbiologically unsafe products entering the food market.

In addition to the issue of pathogen reduction, the GdL and starter culture combination led to a reduced processing time. The more rapid reduction in pH enables quicker and more effective drying.

This study aims to give a new impetus to the exploration of novel ways of obtaining safer products and to the investigation of the potentially synergistic effects of Glucono-delta-Lactone and starter cultures.

Jungbunzlauer can provide additional information about Glucono-delta-Lactone in meat products and further *Listeria* control with Lactates on request.

Glucono-delta-Lactone, a mild fermentation-based acidifier

Glucono-delta-lactone (GdL) is a neutral cyclic ester of gluconic acid formed by the removal of water. Gluconic acid is an organic acid occurring naturally in plants, fruits and other foodstuffs such as wine (up to 0.5%) and honey (up to 1%).

Jungbunzlauer gluconic acid is produced by the aerobic oxidative fermentation of glucose syrup from non-GMO maize. GdL is then prepared by direct crystallization from the aqueous solution of gluconic acid, resulting in a fine, white crystalline powder. GdL is almost odourless and has a slightly sweet taste. Being non-toxic, it is completely metabolised in the body like a carbohydrate.

When added into an aqueous solution, GdL dissolves rapidly into the medium. Its subsequent slow hydrolysis to gluconic acid produces a gentle acidification. The decrease of pH is progressive and continuous to equilibrium, which is outstanding compared to the instantaneous acidification of other acidulants. An addition of 0.1 g/kg GdL will lead to a decrease of around 0.1 pH units in meat matrices. The initial sweet taste of GdL becomes slightly acidic during hydrolysis. However, the final flavour of an aqueous solution of GdL remains much less tart than that of other common food acids (relative sourness of gluconic acid is only 1/3 that of citric and lactic acids and 1/4 that of acetic, malic and tartaric acids). Due to its mild nature, GdL does not mask delicate meat and spice flavours.

Legal aspects of Glucono-delta-Lactone

GdL is a generally permitted food additive (E575) within the European Union. It may be added to all foodstuffs, following the *quantum satis* principle, as long as no special regulation restricts use (Regulation (EC) No 1333/2008).

The US Food and Drug Administration (FDA) assigned "generally recognised as safe" (GRAS) status to GdL and permitted its use in food without limitation other than current good manufacturing practice (GMP) (21 CFR Ch. I §184.1318). Uses of GdL include as a curing and pickling agent, as a pH control agent and as a sequestrant (21 CFR Ch. I §184.1318).

In the International Numbering System of the Codex Alimentarius, GdL has INS number 575 and is categorised as an acidifier/acidity regulator and as a raising agent.

The acceptable daily intake (ADI) of GdL has been established as "not specified" by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Community's Scientific Committee on Food (SCF).

Purity criteria have been laid down for GdL by the main food and pharmaceutical compendia such as the Food Chemicals Codex (FCC), JECFA specifications, Japan's Specifications and Standards for Food Additives (JSFA), the US Pharmacopeia, etc.

For GdL to be used in meat applications, Jungbunzlauer recommends granulation F5010.

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References

[1] Toldrá Fidel: Handbook of Fermented Meat and Poultry, 1st edition, Blackwell Publishing, USA 2007

[2] Internet resource: RASFF Portal European Commission https://webgate.ec.europa.eu/rasff-window/portal/?event=SearchByKeyword&NewSearch=1&Keywords=Salami, last accessed 12.11.2018

[3] Gerhard Feiner: Meat products handbook – Practical science and technology, Woodhead Publishing Limited, England 2006

[4] Martin et al.: Prevalence of Salmonella spp. and Listeria monocytogenes at Small-Scale Spanish Factories Producing Traditional Fermented Sausages, Journal of Food Protection, Vol. 74, No. 5, p. 812-815, 2011

[5] Elliot Ryser and Elmer Marth: Listeria, Listeriosis and Food Safety, 2nd edition, Marcel Dekker, Inc., USA 1999

[6] Delphine Thévenot et al.: Fate of Listeria monocytogenes in experimentally contaminated French sausages, International Journal of Food Microbiology, Elsevier, Vol. 101, Issue 2, p. 189-200, 2005

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Jungbunzlauer offers different granulations of Glucono-delta-Lactone for food applications as well as pharmaceutical and personal care products.

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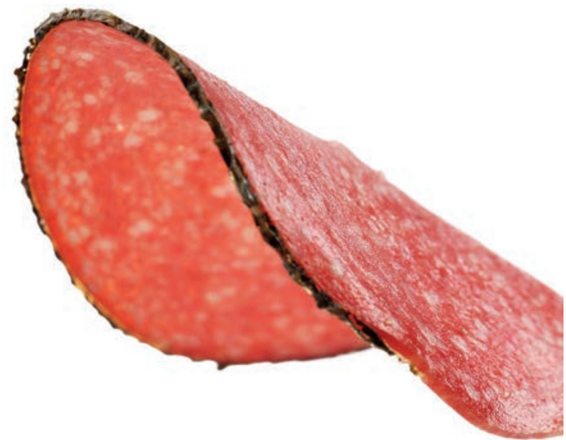

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