**Yersinia enterocolitica** in fermented sausages

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**Abstract.** Different types of food, among them meat, can be the cause of food-borne diseases, and infections are commonly caused by Campylobacter, Salmonella, Yersinia enterocolitica, verotoxic Escherichia coli and Listeria monocytogenes. All these bacteria, depending on a number of factors, including animal species, geographical origin, climatic factors, methods of animal breeding and meat production, could cause disease. Here, we summarise results on production of different groups of sausages produced with or without added starter culture, and contaminated with Y.enterocolitica (control sausages were not contaminated). During the ripening, changes in the microbiological status of the fermented sausages and their physical and chemical properties were monitored. For all tests, standard methods were used. In these fermented sausages, the number of *Y. enterocolitica* decreased during ripening. The number of *Y. enterocolitica* was statistically significantly lower in sausages with added starter culture on all days of the study Zoonotic pathogens in meat should be controlled through the complete production chain, from the farms to consumers, in order to reduce the probability of disease in humans. However, the necessary controls in the production chain are not the same for all bacteria.

1. **Introduction**

Meat processing came about from the need to conserve meat. The ancient Romans conquered the territory of the then known “Old World”, by, among other things, knowing how to conserve meat (they produced dried ham and fermented sausages), so that these products were a source of food of animal origin without the danger of spoiling. Lowering the water activity (a_w), combined with lowering pH, can be considered the oldest meat conservation technology. Dried products were produced by drying in the air, and the a_w was also reduced by salting, whether it was wet or dry salting. Also, in the case of fermented products, salting was used mainly to prevent the growth of bacteria. Little was known about the process of fermentation, the penetration of salt into the meat, or drying. Meat processing was seen as an art, a skill. In those times, smoke was an additional conservation method, especially significant for surface spoilage (mould). Therefore, some importance was given to smoking of meat, which contributes to a more acceptable smell and taste.

2. **Fermented sausages**

Fermented sausages are, in fact, raw sausages, which are prepared from minced meat, solid fat, nitrite salt, sugar and spices. The spontaneous fermentation in this type of sausage is conditioned by the presence of lactic acid bacteria and coagulase negative cocci (including *Kocuria* spp.) in sausage filling (raw materials).
Fermentation is more successfully controlled using starter cultures. *Lactobacillus sakei, L. curvatis, L. plantarum, L. pentosus, L. casei, Pediococcus pentosaceus* and *Pediococcus acidilactici* [1-4] are most commonly used as the starter cultures. After filling into casings, sausages are subjected to ripening, during which there are physical, chemical and enzymatic changes that ensure the shelf life of the finished product, as well as the characteristic sensory properties. Usually, sausage filling intended for the production of sausages of a bigger diameter is coarsely minced, while the filling for smaller diameter sausages is prepared from the meat that is finely minced. In the case of bigger diameter sausages, fermentation processes are more noticeable, while in smaller diameter sausages, the external physical processes, i.e. drying of the product, is more prevailing.

Differences in microbiota, physical and chemical changes occur during ripening, which also affect the quality of the finished product. It is understandable that given the place of production, the production conditions, especially those related to the ripening processes, can be controlled (air-conditioned chambers), i.e. the temperature conditions during ripening, circulation and humidity of air, as well as the time and temperature of smoking can be monitored. Such controlled production conditions apply to both bigger and smaller industrial facilities.

The introduction of glucono-delta-lactone (GdL), or starter cultures, as well as the use of other additives, allowed development of the industrial production of sausages with significantly different properties compared to traditional products [2,3,5,6].

The traditional production of raw fermented sausages, which is quite common in households in Serbia, takes place under uncontrolled conditions (temperature, humidity, fermentation) during the cooler seasons. This process does not rely on starter cultures, but on the activity of fermentative bacteria that are naturally present in the initial microbiota of the meat and environment of the production area; it is these bacteria which start and perform fermentation, i.e. decomposition of carbohydrates present in the filling, mainly to lactic acid [7-9].

In recent years efforts have been made to develop autochthonous starter cultures, i.e. starter culture from microorganisms isolated from these traditional sausages. Such starter cultures should positively affect the safety, while the typical sensory characteristics of traditional sausages remain preserved [10-12].

In many European countries, increased demand for traditional products has been observed. These products are foods with strong regional characteristics originating from non-industrial processes and which are produced in small batches with limited equipment. Preservation of traditional production and authentic products can help small producers and local economies, and it is also important to preserve traditional knowledge, cultural heritage and the regional identity of small and often underdeveloped communities [5,8,10,13,14].

### 3. *Yersinia* in fermented sausages

The genus *Yersinia* belongs to the family *Enterobacteriaceae*. In this genus, 3 of the 12 species are pathogens, among them *Y. enterocolitica*, and 6 biotypes are recognised (5 pathogenic and 1 non-pathogenic). This Gram negative, facultative anaerobe grows in a wide range of temperatures: optimal 28-29°C, minimum -2°C [15]. There have been reported data that *Yersinia* grows at temperatures from -2 to 42°C [16,17] and even at temperatures of below 0°C [18]. It grows in a wide pH range, 4.2-10 and at a minimum aw of 0.96 [15].

It is widespread in nature and can be found in various types of foods, as evidenced by the fact that yersiniosis in humans has been caused by the consumption of milk and milk products, soy products, various types of salads, pork meat products and various ready-to-eat products [19]. After *Salmonella* and *Campylobacter, Y. enterocolitica* occupies third place among the biological pathogens which cause foodborne disease in humans. The number of cases in the European Union in 2011 was 7,017. Probably there were more cases but some were likely not registered (not reported, the cause was not proven). Due to the importance of *Yersinia* in meat, the European Food Safety Authority (EFSA) recommended that in addition to controlling the presence of *Salmonella*, it is mandatory to examine
for the presence of \textit{Y. enterocolitica} in pig carcasses [20]. As \textit{Y. enterocolitica} can frequently be found in pig meat, there is also an understandable interest in its presence in fermented sausages, since they are not thermally treated, and are ready-to-eat.

The number of \textit{Y. enterocolitica} during fermentation of sausages was studied earlier [21,22]. The sausage filling was contaminated with \textit{Y. enterocolitica}, so the number of these bacteria in the filling was 6.17 log CFU/g. The filling was filled into smaller (36 mm) diameter or bigger (50 mm) diameter casings, with subgroups of sausages with or without added starter cultures for both groups. On day 7, the number of \textit{Y. enterocolitica} in smaller diameter sausages, without a starter culture, decreased by 0.87 log CFU/g, and in smaller diameter sausages with added starter culture, decreased by 1.18 log CFU/g from the starting number. In sausages with the bigger diameter, without added starter culture, the number \textit{Y. enterocolitica} on day 18 of ripening decreased by 1.87 log CFU/g, and in sausages with added starter cultures, decreased by 2.10 log CFU/g, from the starting number. In sausages of a smaller diameter, both with and without added starter cultures, \textit{Y. enterocolitica} was not detected on day 12 of ripening, and in sausages with a bigger diameter, on day 25 of ripening.

The authors [21,22] explained the negative finding of \textit{Y. enterocolitica} in sausage by the fact that the number of lactic acid bacteria in the sausages of both diameters was close to or above 9 log CFU/g, that sausage pHs were below 5.5, and the \(a_w\) was 0.93 and 0.95 in smaller and bigger diameter sausages, respectively. In several studies, the possibility of inactivating \textit{Y. enterocolitica} in fermented sausages was examined, whereby the reason for the inactivation was considered the presence of nitrates, starter cultures, pH values, and spice mixtures.

In Turkish fermented sausage, \textit{L. sakei} was used as a starter culture, and \textit{Y. enterocolitica} (about 5 log CFU/g) was inoculated into the sausages [23]. After only three days, the pH was reduced from 6.3 to 4.7 and \textit{Y. enterocolitica} was not found in sausage filling. On the contrary, in the case of sausages without added starter culture (final pH 5.6), \textit{Y. enterocolitica} was confirmed on days 4 and 12 of ripening.

An even more efficient reduction in the number of \textit{Y. enterocolitica} was determined by using \textit{P. acidilactici} starter culture [24]. The number of \textit{Y. enterocolitica} was reduced from 5.1 log CFU/g (day 0) to 1.4 log CFU/g (day 1), so that on days 2 and 3, the number of these bacteria was below 1.0 log CFU/g. After day 4, the presence of \textit{Y. enterocolitica} was not detected in the sujuk sausages. From these results, it can be concluded that the number \textit{L. sakei} or \textit{P. acidilactici} in sujuk sausages completely eliminated \textit{Y. enterocolitica} after just four days of fermentation. The rapid decrease in the number of \textit{Y. enterocolitica} is mostly due to the low pH, i.e. the presence of lactic acid. The presence of salt, nitrite and spice also contributes to the reduction in the number of \textit{Y. enterocolitis} in sujuk sausage [25].

Similar results were obtained by other authors [26]. However, a short period of ripening followed by storage at 8°C could not inactivate \textit{Y. enterocolitica} in sausages. Reduction of \textit{Y. enterocolitica} numbers in sausages is made possible by increasing the temperature and length of ripening [27]. Therefore, \textit{Y. enterocolitica} presents a real danger to human health in fermented sausage spread, where the fermentation time is short, and an extension of sustainability requires a lower storage temperature. In the literature, there are data on the influence of different levels of nitrates and different starter cultures on \textit{Y. enterocolitica} [28], and the activity of \textit{L. sakei}, pH and lactic acid on \textit{Y. enterocolitica} [29].

In traditional dry sausage production, fermentation based on the initial microbiota [9] showed that the number of these microorganisms in the filling of French fermented sausages was, on average, 4.0 log CFU/g, and among them, in addition to the microorganisms useful for fermentation and development of organoleptic properties of sausages (lactic acid bacteria, coagulase negative cocci, yeast and mould), there were also spoilage bacteria (\textit{Pseudomonas} spp., \textit{Enterobacteriaceae}) and enterococci.

The type of microbiota that will develop, dominate or survive in traditional sausages depends on the production recipe, as well as the conditions of fermentation and ripening, and these conditions can be very different in terms of temperature, duration and relative humidity. \textit{Enterobacteriaceae} are
common meat contaminants and, therefore, can be found in filling and sausages in numbers that depend on the initial loading of raw materials, sausage type and phase of ripening. These microorganisms usually are eliminated from fermented sausages during ripening as a result of the combined effects of low pH, low temperatures and low aw values.

The presence of nitrite and lactic acid, as well as other metabolites produced under the influence of starter cultures, further emphasise this phenomenon. Lactic acid bacteria constitute the dominant group of microorganisms after 28 days of ripening, which could be explained by the good adaptation of these microorganisms to the given environment [30,31]. These authors, like others, stated that during the ripening of fermented sausages, the number of all bacterial species decreased except for lactic acid bacteria; these, by producing organic acids and antibacterial substances, had a suppressive effect on Gram negative microorganisms and Y. enterocolitica [6,21,32,33,34,35].

In sausages with the addition of an autochthonous starter culture, the number of bacteria was 3.1 log CFU/g on day 0, 4.6 log CFU/g after the fermentation process and 2.0 log CFU/g on day 50 of production. In control sausages, the number of lactic acid bacteria was 3.0 log CFU/g on day 0 day, 5.1 log CFU/g after the fermentation process and 2.8 log CFU/g on day 50 [10]. According to our results for Enterobacteriaceae [21], these organisms were not detected in control or experimental sausages during later stages of fermentation (in smaller diameter sausages after day 12, and in bigger diameter sausages after day 25).

4. Conclusion

The aim of the fermentation of raw sausages is to obtain safer products, that is, products which do not contain foodborne pathogens which could cause disease, among which is Y. enterocolitica. In fermented sausages that are produced without thermal treatment, during ripening and drying, different parameters are used which could stop/slow the growth of pathogenic bacteria are used, due to the simultaneous action of several factors such as pH decrease, presence of lactic acid, decreases in aw, inhibitory effect of smoke (if the sausages are smoked), presence of starter culture and action of metabolites (bacteriocins), etc.

Inactivation of pathogenic bacteria during the ripening of sausages involves control of their growth and it is a key step in the production of fermented sausages. Reduction of their number ends or is insufficient if the ripening process does not last long enough or is not optimal (incorrect starter culture, temperature, humidity, circulation), which is why this process should be controlled.

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