

## MICROBIOLOGICAL AND PHYSICO-CHEMICAL QUALITY OF ARTISANAL AND INDUSTRIAL DEER SAUSAGES DURING RIPENING

Mariscal Contreras C.; García Ruiz A.; Cabezudo Ibáñez, M.D.

Department of Analytical Chemistry and Food Technology. University of Castilla-La Mancha. Faculty of Chemistry. Campus Universitario s/n. 13071 Ciudad Real. Spain. Fax: 0034 926295318. Tlf. 0034 926295300. e-mail: agruiz@qata-cr.uclm.es

### Background

Deer sausages is a fermented product traditionally made artisanally throughout central Spain. It is prepared from venison mixed with pork back fat, with the addition of pepper, salt, garlic, hot paprika, nitrate, nitrite, and powdered milk protein.

The ingredients are stuffed into artificial casings (50 mm diameter) and ripened at low temperature (10-12 °C) and relative humidity (74-80 %) for about 21 days.

Deer sausage production, especially small-scale production, is still based on the experience and the skill of local manufacturers rather than being based wholly on scientific and technological know-how. In these conditions, the contribution of the native microflora plays a decisive role.

### Objectives

Microbial fermentation is an important process in the manufacture of cured sausages, and it is therefore extremely important to ascertain the population levels reached by the different microbial groups that have technical applications in processing and the trends displayed by such groups over the ripening period. The experiment is also designed to add to our knowledge of the levels and trends for certain other microbial groups that are of interest from the hygienic and sanitary standpoints.

The microbial species present will later be identified. The results of the study are expected to be of considerable use in obtaining an industrial product of high, uniform quality.

### Methods

#### Samples

Two batches of deer sausages were made, one home-made (in a natural dryer) and one industrial (in dryers with regulated temperature and humidity). Samples of the venison and the sausages were taken from each batch at 0, 7, 14, and 21 days of ripening.

#### Physicochemical analysis

Water activity ( $a_w$ ) was determined using a dew-point hygrometer (Decagon Devices, model CX-2). The pH was measured using an Ingold electrode probe connected to a Crison model 2001 pH-meter. Moisture was determined using ISO method R-1442 (ISO, 1973).

#### Microbiological analysis

For microbiological analysis, the casings were removed aseptically. Ten grams of sausage were homogenized in a stomacher in 90 ml of 2 % (w/v) sodium citrate for two minutes. Decimal dilutions of the suspension were prepared in sodium citrate and spread on the appropriate plates. The aerobic mesophilic flora was counted on Standard Plate Count Agar (Cultimed) after incubation at 30 °C for 72 h; lactic acid bacteria in M.R.S. Agar (Difco) after incubation at 30 °C for 24 h; micrococci and staphylococci on Salt Mannitol Agar (Difco) after incubation at 37 °C for 72 h; enterococci in Kanamycin Aesculin Azide Agar (Oxoid) (Mossel *et al.*, 1978) after incubation at 37 °C for 48 h; and Baird Parker (Difco) were the media of choice for *Staphylococcus aureus* after incubation at 37 °C for 48h. *Enterobacteriaceae* were enumerated in Violet Red Bile Glucose agar (Difco) after incubation at 37 °C for 24 h; coliforms in Violet Red Bile Agar (Difco) after incubation under anaerobic conditions at 37 °C for 24 h; moulds and yeasts were counted in Potato Dextrose Agar (Difco) pH=4.6 after incubation at 26 °C for 96 h.

*Salmonella* were assayed using ISO method 3565 (ISO, 1983), and *Listeria monocytogenes* were enumerated on selective Palcam agar (Merck), with plates being incubated aerobically at 37 °C for 48 h.

### Results and discussion

Tables 1 and 2 show the changes in the microbial groups studied during ripening of the batches of deer sausages made industrially and artisanally (home-made), respectively. The industrially manufactured batch had higher counts of mesophilic flora than the artisanal batch. Neither *Salmonella* nor *Listeria monocytogenes* were detectable in either of the batches tested (absent in 25 g).

The trends for the different microbial groups and the counts for each one were basically similar to those observed in such other fermented sausages as salchichón (Sanz *et al.*, 1997), chorizo de León (Dominguez *et al.*, 1989) and salami (Samelis *et al.*, 1994).

Lactic acid bacteria were the dominant microflora in the two batches studied and displayed normal behaviour.

Levels of *Enterobacteriaceae*, enterococci, and coliforms gradually fell with decreasing  $a_w$  in both batches (Figures 1 and 2).

There were differences between the artisanal and industrial batches. Lactic acid bacterial growth during the first few days of ripening seemed to be faster in the artisanal batch, with the industrial batch having higher lactic acid bacterial counts towards the end of ripening (Figure 3). The growth of the lactic acid bacterial population helped give rise to suitable conditions (decreasing pH) for inhibiting the growth of other microbial groups (*Enterobacteriaceae*, coliforms, and enterococci).

The steepest decline in pH in the two batches took place during the first week of ripening (data not shown). The pH was slightly higher in the artisanal batch throughout the manufacturing process.

The pH was negatively correlated to the lactic acid bacterial counts in both batches (artisanal  $r=-0.951$ ;  $p<0.05$ , industrial  $r=-0.762$ ;  $p<0.05$ ).

### Conclusions

No pathogenic microorganisms such as *Salmonella* or *L. monocytogenes* were detected in either the artisanal or the industrial deer sausages.

Microbial counts were high, as a rule, attributable primarily to the conditions of collection and transport of venison.

The  $a_w$  decreased more sharply in the industrial deer sausage batch on account of the controlled temperature and relative humidity conditions, which doubtless exerted an effect on the growth of the different microbial groups.

### Acknowledgements

The authors gratefully acknowledge the financial support for this study in the form of a research grant provided by the Government of the Castilla-La Mancha Autonomous Region.

**References**

- Dominguez et al. (1989). *Alimentaria* 199, 11-15.
- Ferreira Vacas, J. (2001). Tesina de Licenciatura. Facultad de veterinaria. Universidad de Cordoba. Cordoba, España
- ISO METHOD 1442 (1973). International Standards Organization. Geneva. Switzerland.
- ISO METHOD 3565 (1983). International Standards Organization. Geneva. Switzerland.
- Mossel, D.A.A. et al. (1978). *Arch. Lebensmittelhyg.*, **29**, 121-127.
- Samelis et al. (1997). *Food Microbiol.* **11**, 447-460.
- Sanz et al. (1997). *Food Microbiol.* **14**, 575-582.

Table 1: Changes in the different microbial groups (log cfu./g) during the ripening of deer sausages manufactured industrially

	Venison	Day 0	Day 7	Day 14	Day 21
Aerobic mesophilic flora	5,53 ± 0,12	6,70 ± 0,13	9,21 ± 0,03	9,28 ± 0,03	9,15 ± 0,09
Lactic acid bacteria	4,93 ± 0,04	5,97 ± 0,08	6,00 ± 0,00	8,80 ± 0,04	9,02 ± 0,02
<i>Micrococcus</i> and <i>Staphylococcus</i>				4,73 ± 0,02	4,97 ± 0,00
<i>Staphylococcus aureus</i>	4,7 ± 0,37	5,64 ± 0,01	4,54 ± 0,05	3,92 ± 0,32	4,52 ± 0,06
<i>Enterobacteriaceae</i>	4,33 ± 0,13	4,22 ± 0,00	4,43 ± 0,01	3,08 ± 0,18	3,08 ± 0,05
Coliforms	4,27 ± 0,22	3,8 ± 0,00	4,24 ± 0,21	2,85 ± 0,21	1,00 ± 0,00
Enterococci	4,94 ± 0,1	4,86 ± 0,06	6,17 ± 0,02	3,50 ± 0,04	3,8 ± 0,08
Moulds and yeast	6,12 ± 0,35	4,67 ± 0,24	6,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00

Table 2: Changes in the different microbial groups (log cfu./g) during the ripening of deer sausages produced artisanally

	Venison	Day 0	Day 7	Day 14	Day 21
Aerobic mesophilic flora	5,7 ± 0,09	5,11 ± 0,05	7,69 ± 0,06	7,88 ± 0,01	8,2 ± 0,01
Lactic acid bacteria	3,83 ± 0,18	3,84 ± 0,16	7,30 ± 0,15	7,90 ± 0,23	7,91 ± 0,02
<i>Micrococcus</i> and <i>Staphylococcus</i>			4,53 ± 0,08	5,99 ± 0,03	6,26 ± 0,00
<i>Staphylococcus aureus</i>	5,5 ± 0,09	4,93 ± 0,02	5,94 ± 0,08	6,4 ± 0,01	6,48 ± 0,04
<i>Enterobacteriaceae</i>	4,48 ± 0,04	4,52 ± 0,01	4,16 ± 0,22	3,24 ± 0,09	3,26 ± 0,01
Coliforms	4,05 ± 0,60	4,51 ± 0,06	4,24 ± 0,10	2,85 ± 0,59	2,96 ± 0,06
Enterococci	4,54 ± 0,13	2,85 ± 0,00	3,1 ± 0,14	2,30 ± 0,43	3,23 ± 0,00
Moulds and yeast	5,40 ± 0,02	4,09 ± 0,16	5,89 ± 0,10	7,08 ± 0,15	6,00 ± 0,00

Figure 1: Changes in the counts (log cfu/g) of certain microbial groups and a<sub>w</sub> in artisanal deer sausages during ripening

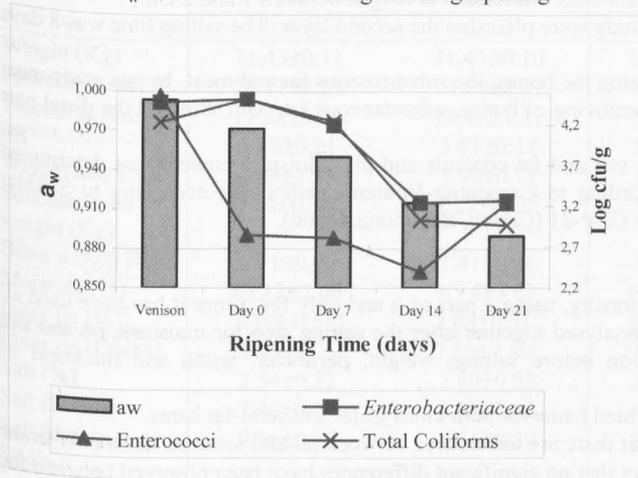


Figure 2: Changes in the counts (log cfu/g) of certain microbial groups and a<sub>w</sub> in industrial deer sausages during ripening

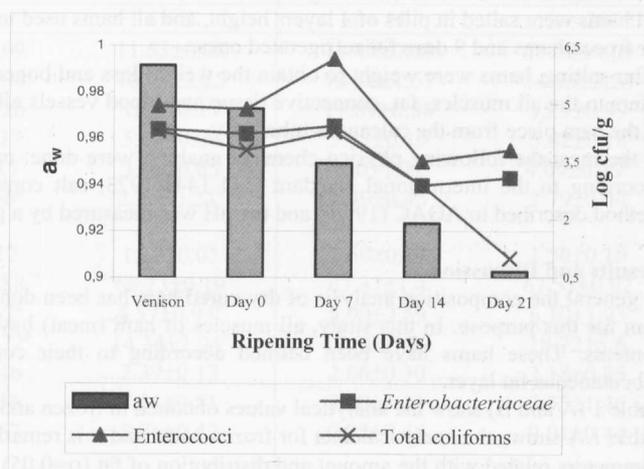


Figure 3: Changes in lactic acid bacterial counts (log cfu/g) in deer sausages during ripening

