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Effect of probiotic bacteria on chemical composition and sensory quality of fermented sausages

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Abstract

Probiotic food products are very popular on domestic and international markets. The application of probiotics in dairy products is quite frequent, while their application in meat products is still being explored. Three variants of Sremska-type fermented sausages were produced using starter culture Bactoform T-SPX (Chr. Hansen): 1. control sausage variant; 2. variant with *Lactobacillus helveticus* RO52 (Lallemand, France); 3. variant with *Bifidobacterium longum* RO175 (Lallemand, France). Through 40 days of fermented sausage ripening, the survival of probiotic bacteria, the changes of starter bacteria counts, as well as the chemical composition, pH values and sensory evaluation were examined. During the first period of ripening, *Lactobacillus helveticus* RO52 and *Bifidobacterium longum* RO175 counts were 10^6 cfug⁻¹, after which they increased to the level of 10^8 cfug⁻¹ and remained there until the end of ripening. Starter bacteria counts were within the range typical for fermented sausages. The chemical composition and pH values of fermented sausages produced with probiotic bacteria did not significantly differ from the control variant. Sensory evaluation has shown that all variants of fermented sausages had an acceptable sensory quality. Based on the survival of probiotic bacteria during 40 days of fermented sausage ripening, it can be concluded that probiotics can be successfully used in the production of fermented sausages, without affecting the sensory quality of the sausage.

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1. Introduction

Probiotic lactic acid bacteria are living micro-organisms which have a beneficial effect on the health of the consumer when ingested in certain amounts. Consumption of probiotics has a positive effect on the intestinal microflora, colonization resistance against pathogens and shows beneficial immune responses [13]. Probiotics are widely used in dairy products, but their application in meat products is still being explored. Meat is generally heated before consumption, which kills probiotic bacteria, but dry sausages are processed by fermenting, without heating, and can be good media for probiotic application in meat products [4]. In addition, the sausage matrix protects the survival of probiotic lactobacilli through the gastrointestinal tract [10]. Anderssen [1] investigated the application of a potentially probiotic strain *L. casei* LC-01 in mixture with starter culture Bactoferm T-SPX (Chr. Hansen) or probiotic *Bifidobacterium lactis* Bb-12 in mixture with same starter, in dry sausages production. The commercial meat starter strains *Lactobacillus sakei* Lb3 and *Pediococcus acidilactici* PA-2 may be interesting because of their survival capacities under simulated gastrointestinal conditions [2]. Apart from contributing to human health, probiotic fermented sausages need to be of sufficient commercial value. The primary requirement remains the sensory technological aspects of the end-product. Probiotic strains *L. rhamnosus* GG, *L. rhamnosus* LC705, *L. rhamnosus* E-97800 and *L. plantarum* E-98098 do not negatively affect the technological or sensory properties of the end-product [3]. The aim of this work was to investigate survival of probiotic bacteria and their effect on the basic chemical composition and sensory quality of dry fermented sausages.

2. Materials and Methods

2.1. Sausages manufacture and sampling

Ten kilograms of sausages batter was prepared from fresh meats purchased from a local wholesaler. Pork and pork fat at 75% and 25% were frozen, tempered to -2°C, chopped and mixed in a rotating bowl meat cutter (Seydelman K60, Germany) to 8mm. The sausages batter was inoculated with a commercial meat starter Bactoferm T-SPX (Chr. Hansen) consisting of a mixture of *Pedococcus pentosaceus* and *Staphylococcus xylosus*, according to instructions by the producer (variant 1), a commercial starter Bactoferm T-SPX (Chr. Hansen) + *Lactobacillus helveticus* RO52 (Lallemand, France) aiming at 6 log cfug⁻¹ (variant 2), a commercial starter Bactoferm T-SPX (Chr. Hansen) + *Bifidobacterium longum* RO175 (Lallemand, France) aiming at 6 log cfug⁻¹ (variant 3). The same amounts of ingredients were added to all sausage variants: 2.3 % salt, 0.011 % NaNO₂, 0.3 % dextrose, 0.20 % garlic and 0.5 % sweet red paprika. The mixture was filled in natural casings made of pig intestines of 32mm. After filling, the sausages were hung on sticks and the ripening was carried out in a drying chamber under controlled conditions (Maurer, Germany) and under the following regime: day 1 – relative humidity (RH) 90 % at 21°C, day 2 RH 88 % at 20 °C with smoking, day 3 RH 85 % at 20 °C; during the following days RH was reduced by 1 % on a daily basis and the temperature was constant at 16 °C.

Sampling was carried out on production days 0, 3, 7, 14 and storage days 40. After 14 days the sausages were packed in vacuum bags and stored at 4–7 °C up to 40 days.

2.2. Chemical analysis

The chemical composition of meat was determined in the following manner: water content by drying samples at 105 °C [7]; protein content by Kjeldahl method and multiplying by factor 6.25 [6]; fat content by Soxhlet method [8], and ash content by sample mineralization at 550–600 °C [5]. NaCl content was established by Volhard method [9].

2.3. pH values

The pH value was measured by pH-meter Hanna, HI 83141 (Hanna Instruments USA). The presented results are mean values of three measurements.

2.4. Microbiological analysis

Two 10g slices from each sausage sample were weighed aseptically, transferred to sterile saline diluents containing 1 % peptone and homogenized for 2 min using Stomacher 400 (Seward, London, UK). Appropriate decimal dilutions of the samples were prepared using the same diluents and plated in duplicate on different growth media. Starter bacteria counts were determined as follows: *Pediococcus pentosaceus* on MRS agar (Oxoid, CM 0361) + polymixin B (0.15gL^{-1}) and *Staphylococcus xylosus* on mannitol salt phenol-red agar (MSA, Oxoid, CM 0085) at $37\text{ }^{\circ}\text{C}$ for two days. Probiotic bacteria were selectively enumerated *Lactobacillus helveticus* RO52 on MRS agar + ciprofloxacin (20mgL^{-1}) and *Bifidobacterium longum* RO175 on MRS agar supplemented with glucose (20gL^{-1}) and dichloxallin (0.5mgL^{-1}), lithium chloride (1gL^{-1}), cystein hydrochloride (0.5gL^{-1}). Plates were microaerophilic incubated in Gas Pack (BBL, Germany) at $30\text{ }^{\circ}\text{C}$ for 3 days. Microbiological data were transformed into logarithms of the number of colony-forming units (cfug^{-1}).

2.5. Sensory analysis

The evaluation of sensory characteristics of sausages was conducted by eight assessors with previous experience in the evaluation of dried fermented sausages. Sausage samples were evaluated after 14 and 40 days from the beginning of production. Prior to each evaluation, preparatory meetings were held to discuss in detail the defined characteristics of sausages which were to be evaluated. A nine-points system was used to evaluate the appearance, cross section, colour, odour, texture and taste of sausages (1 – extremely unacceptable, 9 – extremely acceptable). The presented data are mean values of eight evaluations.

3. Results and Discussion

3.1. Chemical composition

Changes in basic chemical components of experimental sausages during ripening are shown in Table 1. At the beginning, in variants 2 and 3 moisture content was some lower (54.09 and 55.53%) than in variant 1 (58.44 %), but during the storage, on 40th day, moisture content was similar in all samples (about 24–25 %). These results are in line with the data for similar dry fermented sausages in Greece, Hungary and Croatia [11]. Contrary to that, in samples 1 on day 0, fat content was the lowest (15.50 %), while in variants 2 and 3 it was 19.82 % and 18.17 %, but at the end of storage in all variants fat content were about 41 %.

Table 1. Chemical composition of sausages during ripening and storage

Sausages	Percent (%)	Days				
		0	3	7	14	40
1. Variant	Water	58,44	45,84	38,49	27,64	24.24
	Salt	2,61	3,28	4,02	4,79	4.86
	Fat	15,50	26,29	36,59	41,33	40.88
	Total protein	17,93	19,22	19,56	26,28	27.21
2. Variant	Water	54,09	46,74	35,01	27,86	24.19
	Salt	2,60	3,31	4,01	4,46	5.11
	Fat	19,82	29,91	37,76	41,80	41.38
	Total protein	17,99	18,37	21,09	25,77	26.83
3. Variant	Water	55,53	47,43	33,80	26,66	24.93
	Salt	3,10	3,55	4,13	4,75	5.52
	Fat	18,17	27,90	40,70	42,28	41.46
	Total protein	18,15	18,05	19,84	24,31	25.92

At the beginning, total protein content in all variants was about 18 % and at the end of storage it was about 27 %. Chemical composition was similar in all variants of experimental sausages within the range typical for fermented dried sausages.

Changes in pH values are shown in Fig. 1.

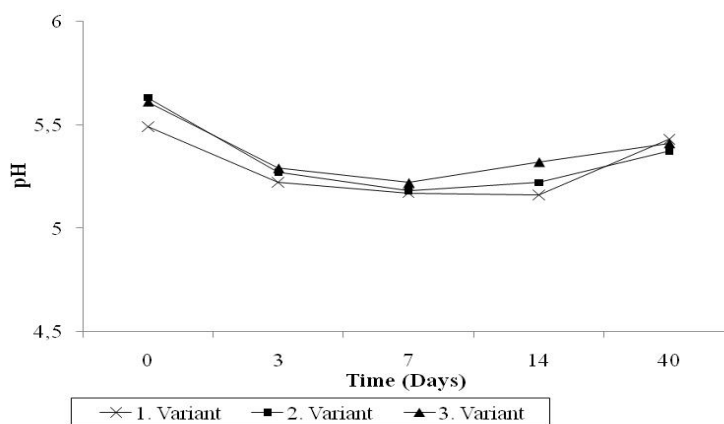


Fig. 1. Changes in pH values during ripening and storage of dry fermented sausages

Initial pH values ranged from 5.49 to 5.63 and 5.61 in samples 1, 2 and 3 respectively. Over the next seven days of ripening, the pH gradually dropped in all samples and reached the minimum value of 5.17–5.22 on day 7. On day 14 the pH values were 5.32–5.16 and at the end of storage, on day 40, the pH values were 5.37–5.43. The dynamics of these changes was similar in all samples.

3.2. Microbiological analysis

Results of a microbiological analysis are presented in figures 2–3. Initial *P. pentosaceus* counts were approximately $6 \log \text{cfug}^{-1}$ for all samples. The level of *P. pentosaceus* increased by 2 log units during 3 days of drying and reached $8 \log \text{cfug}^{-1}$, and then stagnated on that level up to 7th day of ripening (Fig.2). A slight decrease ($0.5 \log \text{cfug}^{-1}$) was detected at the end of drying, and after the storage period in vacuum

conditions, the level slightly increased and reached nearly of $8 \log \text{cfug}^{-1}$. The high level of *P. pentosaceus* during the manufacture of dry fermented sausages was detected by Muthukumarasamy et al. [12]. *S. xylosus* numbers maintained on the level of $6 \log \text{cfug}^{-1}$ during 14 days of drying, and during the storage they increased to the level of about $1 \log \text{cfug}^{-1}$ in all treatments. Similar trends were detected in dry fermented sausages produced with *S. carnosus* [12].

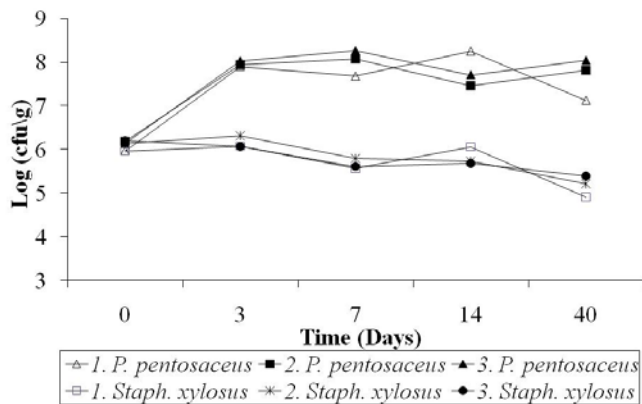


Fig. 2. Survival of *P. pentosaceus* and *S. xylosus* during ripening and storage of dry fermented sausages

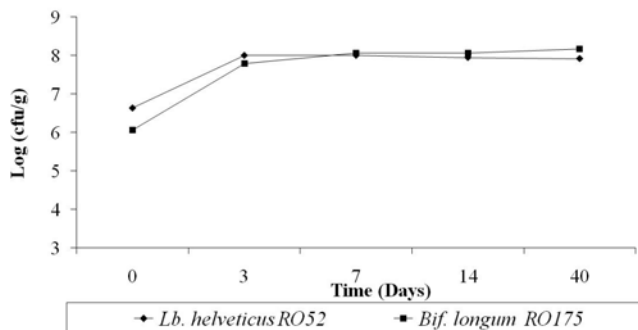


Fig. 3. Survival of probiotic bacteria during ripening and storage of dry fermented sausages

In both variants (2, 3) of probiotic sausages, the initial counts of probiotic bacteria were approximately $6 \log \text{cfug}^{-1}$ (Fig.3). During 3 days of sausage drying, the probiotic bacteria counts increased by 2 log units and maintained on the level of $8 \log \text{cfug}^{-1}$ until the end of the storage period. These values met the requirement for probiotic number to achieve a healthy effect. The application of probiotics in dry fermented sausages depends on their ability to survive during sausage processing. The potential for dry fermented sausages to serve as a vehicle for probiotic bacteria has been reviewed by Työppönen et al [13].

3.3. Sensory analysis

Fermented sausages were evaluated on days 14 and 40. Results of sensory evaluation are presented in Fig. 4–5.

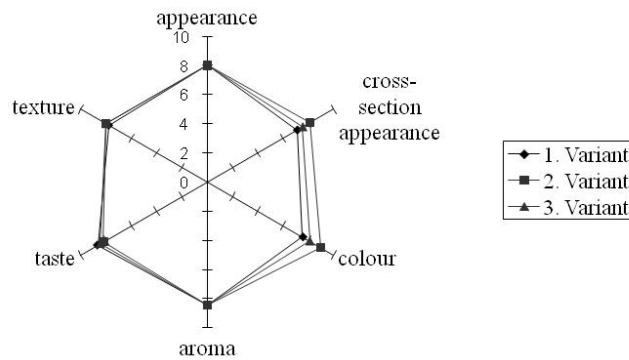


Fig. 4. Sensory analysis of dry fermented sausages at the end of ripening (14 days)

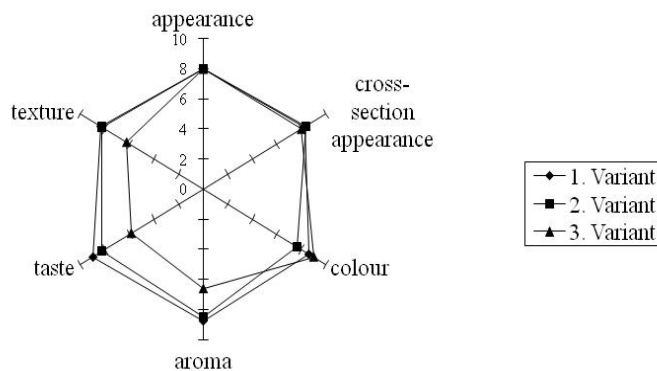


Fig. 5. Sensory analysis of dry fermented sausages at the end of storage (40 days)

After 14 days, all quality parameters were evaluated relatively high, with grades over 7, without significant differences between sausage variants. After 40 days, variants 1 and 2 were very similar with grades over 7.6, but variant 3 received somewhat lower grades for aroma (6.75), taste (6.0) and texture (6.25). All samples were evaluated as acceptable.

4. Conclusion

It can be concluded that probiotic *Lactobacillus helveticus* RO52 and *Bifidobacterium longum* RO175 (Lallemand, France) seem to be suitable for the production of dry sausages. The survival of probiotic bacteria was on a high level of 8 log cfug-1 at the end of storage period in both probiotic sausage variants. There were no significant differences in starter bacteria counts. Chemical composition and pH values were similar in control and probiotic samples of sausages. Sensory quality was acceptable in all sausage variants, but somewhat better aroma, taste and texture were detected in sausages produced with *Lactobacillus helveticus* RO52.

Acknowledgments

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References

- [1] Anderssen L. Fermented dry sausages produced with the admixture of probiotic cultures. 44th *International congress of „Meat science and technology”* 1998; 826.
- [2] Erkkilä S, Petäjä E. Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. *Meat Sci* 2000; **55**: 297-300.
- [3] Erkkilä S, Petäjä E, Eorola S, Lilleberg L, Mattila-Sandholm T, Suihko ML. Flavour profiles of dry sausage fermented by selected novel meat starter cultures. *Meat Sci* 2001; **58**: 111-116.
- [4] Erkkilä S, Suihko ML, Eerola S, Petäjä E, Mattila-Sandholm S. Dry sausage fermented by *Lactobacillus rhamnosus* strains. *Int J Food Microbiol* 2001; **64**: 205-210.
- [5] ISO 936. Meat and meat products. Determination of total ash. In: *International organization for standardization* 1998. Geneva.
- [6] ISO 937. Meat and meat products. Determination of nitrogen content (reference method). In: *International organization for standardization* 1978. Geneva.
- [7] ISO 1442. Meat and meat products. Determination of moisture content (reference method). In: *International organization for standardization* 1997. Geneva.
- [8] ISO 1443. Meat and meat products. Determination of total fat content (reference method). In: *International organization for standardization* 1973 . Geneva.
- [9] ISO 1841-1. Meat and meat products. Determination of chloride content- Part1: Volhard method (reference method). In: *International organization for standardization* 1996. Geneva.
- [10] Klingberg TD, Budde BB. The survival and persistence in the human gastrointestinal tract of five potential probiotic lactobacilli consumed as friez-dried culture or as probiotic sausage. *Int J Food Microbiol* 2006; **109**: 157- 159.
- [11] Kozacinski L, Drosinos E, Caklovica F, Cocolin L, Gasparik-Reichardt J, Veskovic S. Investigation of microbial association of traditionally fermented sausages. *Food Tech and Biotech* 2008; **46**: 93-106.
- [12] Muthukumarasamy P, Holley RA. Microbiological and sensory quality of dry fermented sausage containing alginate-microencapsulated *Lactobacillus reuteri*. *Int J Food Microbiol*; **111**: 164-169.
- [13] Salminen S, Deighton M, Benno Y, Gorbach S. *Lactic acid bacteria in health and disease*. 2nd ed. New York: Marcel Dekker; 1998.

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