Fermented, dry sausages produced with the admixture of probiotic cultures

M Sc. Food Science Lone Andersen, Chr. Hansen A/S, 10-12 Bøge Allé, DK-2970 Hørsholm, Denmark

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Introduction

In the LABIP-workshop probiotic, 1995, the definition of probiotics was established: "Oral probiotics are living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition. Probiotics can be consumed either as a food ingredient or as nutraceuticals" (Hammes und Haller, 1998). From its emergence in anecdotal reports the evidence that is currently accumulating seems to indicate that a few well-characterised lactic acid bacteria (LAB) have health promoting effects and as such they are promising ingredients in new functional foods (Salminen *et al*, 1996).

In humans the intestinal microflora is mostly located in the large bowel and attains population levels of approx. 10^{10} CFU/g wet weight. The human colon, for example, contains at least 10^{12} living bacterial cells. Of the about 400 bacterial species present in human faeces 30 to 40 species, including *Lactobacillus acidophilus* and *Bifidobacterium lactis* in small quantities, constitute 99% of the complex composition (Tannock, 1997). The normal, human gastrointestinal microflora is usually stable with interpersonal composition variations. Regardless, the ecosystem may be disturbed by factors such as antimicrobial therapy, diet, pathologic conditions, pelvic radiotherapy and immunocompromised persons (Black *et al*, 1991). Tannock (1997) put forward the view that ingested viable probiotic bacteria would have an impact on the composition of the microflora of the intestinal tract whether it is to stabilise the normal flora or improve an abnormal gut flora. It is also acknowledged that the beneficial effect of probiotics requires a continuous intake of these bacteria in the diet (Mogensen, 1995). A total daily intake of at least 10^8 probiotic bacteria is normally recommended.

The concept of probiotic products is widely used within the dairy industry with the addition of for example *Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium lactis* to frozen yoghurt, fermented and non-fermented milk, cheese, butter, infant formula and dry powders (Hansen, 1997). With reference to the world-wide trend towards new probiotic functional foods and the additive effect of obtaining a sufficient daily intake of probiotics, meats may be another application area. The probiotic strains are, however, known to be sensitive to salt and therefore it is not quite so obvious to apply the probiotics to meat products. Especially not to fermented, dry sausages with a high salt-in-water level.

Objective

The present study was undertaken to explore the possibilities of interpolating the probiotic concept into the product range of fermented sausages. Sausages were produced with the starter culture Bactoferm T-SPX (consisting of *Staphylococcus xylosus* DD-34 and *Pediococcus pentosaceus* PC-1) and the commercially available probiotic strains *Lactobacillus acidophilus* La-5, *Lactobacillus casei* LC-01, and *Bifidobacterium lactis* Bb-12, respectively. As the ability to survive as living bacteria is crucial for added probiotic strains, analytical methods were needed to enumerate these strains separately.

Materials and methods

Two sausage productions were carried out using the following recipe: 31.5 % of beef and pork meat each (approx. 15% fat), 31.5 % back fat, 1.7 % nitrite salt (with 0.6% nitrite), 1.2% potato starch, 1.0 % salt, 0.7 % maltodextrin, 0.4% dextrose/glucose, 0.05 kg Na-ascorbate and a spice blend (white pepper and garlic powder). Furthermore, a mince of one of the three probiotic strains of approx. $5x10^6$ CFU/g and 0.025% Bactoferm T-SPX was applied. A control code with no bacteria added and a code with T-SPX were included. The sausages were processed in a computer controlled Multimat climate chamber in accordance with a traditional three weeks climate chamber programme. After 20 days the sausages were vacuum-packed and further matured at 5°C.

The five sausage codes in each production were explored with respect to pH in the sausages, weight loss and bacteriological examination. One sample of one sausage was analysed on the day of production and subsequently analysed on day 1, 2, 3, 6, 13 and 20.

The bacteriological examination was carried out with a sample size of approx. 40 g, diluted ten times with sterile peptone water and treated for two minutes in a Stomacher. LAB were detected on MRS (Oxoid), anaerobically incubated, for three days at 30°C. Furthermore, the probiotic strains were enumerated separately as follows: La-5 on MRS added 112 mg Moxalactam/l (Sigma M-8158), LC-01 on MRS added 0.5 mg Clindamycin/l (Sigma C-5269), and Bb-12 on MRS added 60 mg Gentamycin/l (Merck 11977), 100 mg Oxgall/l (Difco 0128-15) and 500 mg Cystein/l (10% CyHCL solution, CH-0398). The plates were anaerobically incubated, for three days at 37°C. The recovery was 100% except for Bb-12 of which 50% was found. All cell counts were done by pour plating (enumeration) as well as spread plating (visual recognition). For verification purposes, a proportion of each colony type deviating in colony morphology was microscopically examined.

Sensory assessments by an internal expert panel in respect of texture, appearance and flavour were carried out after two weeks of storage at 5°C.

Results and discussion

The raw materials are of an acceptable bacteriological quality and the development in pH and weight loss are as expected (data not shown). The data from the two sausage productions show a batch to batch variation as could be expected, primarily due to differences in the quality of the raw materials. Furthermore, it should be considered that the development in pH, and therefore also in weight loss, should be viewed as relative developments. The addition of a starter culture does not result in the same pH profile when used in different recipes, production procedures or countries.

The development in LAB is similar in all codes and it is depicted in figure 1. After the third day of processing they have reached approx. 10^8 CFU/g and stay at this level. The microscopic examination reveals that PC-1 from the starter culture dominates (more than 75% of the flora) all through the processing. In figure 2 the survival of the probiotic bacteria added is illustrated. LC-01 and Bb-12 grow approx. one log unit during the first three days and remain at that level during the remainder of the processing period. On the other hand, La-5 does not survive the processing very well, probably due to the increasing salt content during the drying out. The developed substrates' (antibiotics in MRS) efficiency to suppress other LAB than the expected probiotic strain is confirmed by the microscopic examinations.

The pH development in the sausages (figure 3) is similar in the codes with SPX, SPX + La-5 and SPX + Bb-12, whereas the codes with SPX + LC-01 obtain a lower final pH. The difference in the pH development does not result in differences in weight loss, which is illustrated in figure 4. Here the batch to batch variation is larger than the variation between the different codes in a batch.

The sensory assessments show that sausages with a starter culture and with/without the three probiotic strains separately added all give good aromatic sausages. There are no obvious differences in colour, texture, and cohesion between the four codes. Only minor differences are observed between the flavour of the sausages where T-SPX + LC-01 give a higher intensity of sourness compared to the others.

Conclusion

The concept of probiotic, fermented, dry sausages has been tested. The production of fermented sausages with the probiotic strains *Lactobacillus casei* LC-01 and *Bifidobacterium lactis* Bb-12 in combination with a traditional starter culture as Bactoferm T-SPX is possible. Both probiotic strains develop and survive the processing at an acceptable level. Whereas *Lactobacillus acidophilus* La-5 does not survive the processing and therefore it is not recommendable. All three probiotics do not influence the flavour negatively while LC-01 results in a flavour with more sour notes.

References A list of the references used is available upon request.

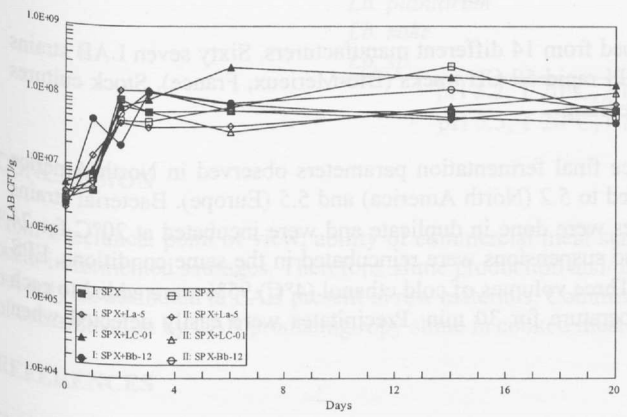

Figure 1. Development of LAB in fermented sausages

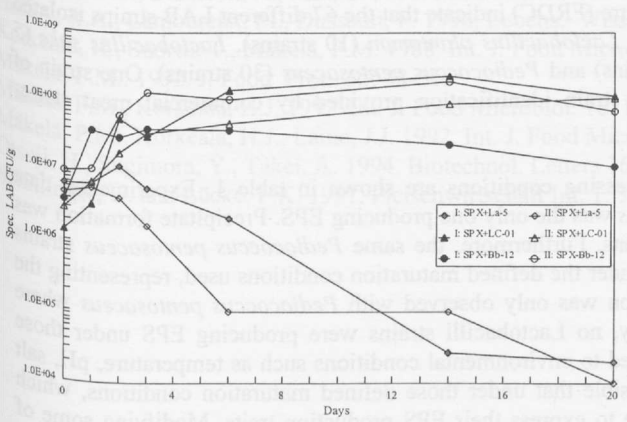

Figure 2. Development of probiotic strains in fermented sausages

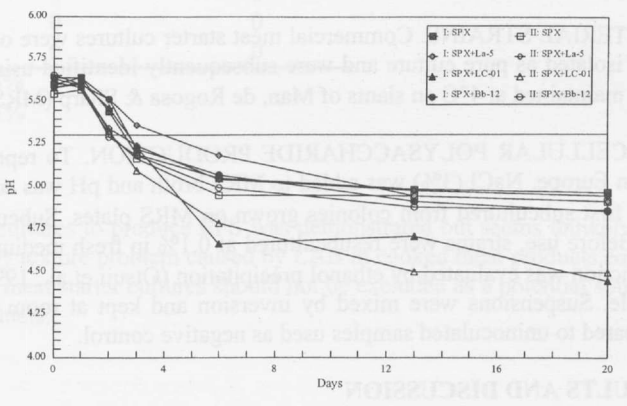

Figure 3. Development in pH in fermented sausages

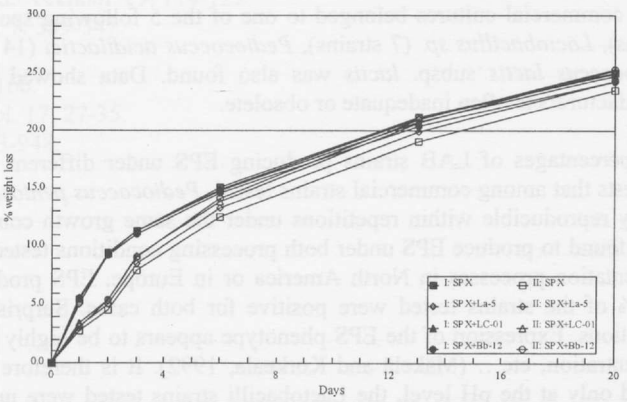

Figure 4. Development in weight loss in fermented sausages