PE9.09 Properties of dry fermented sausages with the addition of probiotic and prebiotic 57.00

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***Abstract* — The aim of this study was the development of a dry sausage with functional properties and a suitable sensory quality. Three groups of dry fermented sausages were prepared, all with a commercial meat starter culture (Bactoferm SM 181, *Lactobacillus sake* HJ-7 and *Staphylococcus xylosus* DD-34): (I) starter culture alone; (II) starter culture plus the probiotic *L. paracasei* subsp. *Paracasei* and a prebiotic (inulin); and (III) starter culture, the probiotic and the prebiotic, plus a reduced fat content. Addition of the probiotic *L. paracasei* subsp. *paracasei* (1.25 × 10^7 CFU/g of sausage) and the prebiotic inulin (1%) had no negative effects on the sensory properties. Their addition to the basic starter culture did not affect pH and water activity during fermentation of the sausage. A reduced fat content from 35% to 28% in combination with the standard meat culture, the probiotic and the prebiotic (group III) did not alter the sensory quality and improve the nutrition value, due to the lowest energy value. Dry fermented sausages are an appropriate medium for probiotics, as *L. paracasei* subsp. *paracasei* survived throughout the fermentation, ripening and two-month storage at 8 °C to 10 °C, and was present in the final product at the predicted level of 10^7 CFU/g or more.**

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***Index Terms* — dry fermented sausage, probiotics, prebiotics, low fat product.**

I. INTRODUCTION

Probiotic products are increasingly important functional foods [1]. Probiotic bacteria have to meet requirements for safety, and functional and technological properties, as well as to survive the manufacturing and storage processes. For successful incorporation into food, they must not produce off-flavours, nor adversely affect the sensory properties of the food [2,3,4].

The aim of this study was to define the effects of addition of a probiotic and the prebiotic inulin, and of a modified reduced fat content on the quality parameters of dry fermented sausages. No significant negative influences of these factors were expected on the sensory properties; rather, a positive influence on the nutritional parameters was expected.

II. MATERIALS AND METHODS

Three groups of dry fermented sausages were compared: (I) with a commercial meat starter culture (Bactoferm SM 181 (Chr. Hansen) containing *Lactobacillus sake* HJ-7 and *Staphylococcus xylosus* DD-34); (II) with the commercial meat starter culture, plus the probiotic *L. paracasei* subsp. *paracasei* and the prebiotic inulin; and (III) with the commercial meat starter culture, the probiotic and the prebiotic, and with a modified composition (reduced fat content).

Throughout the fermentation and ripening, the physical parameters were monitored, with the pH measured on the day of filling into the collagen casings, and after 3, 7, 14, 28 and 35 days, and the weight loss of the products also monitored. At the end of ripening, the water activity (a_w) was measured, and chemical analyses were carried out for water, fat, protein, minerals, salt [5] and inulin [6] contents. The energy values (kJ) of the dry fermented sausages were calculated per sample of 100 g, using the following values: fat, 38.9 kJ/g; protein, 17.1 kJ/g; and carbohydrate, 17.1 kJ/g; the energy value of inulin was estimated at 6.3 kJ/g. The sensory properties were evaluated (scoring according to a non-structured scale from 1 to 7 points, where a higher score means more expression of a property) [7], and instrumental analyses of the texture were performed using a Texture analyser TA.XT apparatus with a Volodkevich bite jaw and a Kramer shear cell.

Microbiological analyses included total cell counts, number of lactic acid bacteria, and number and survival of the probiotic L. *paracasei* subsp. *paracasei*, and they were performed at the end of ripening and after the two-month storage period at 8 °C to 10 °C. To confirm the survival of *L. paracasei* subsp. *paracasei*, DNA analysis was carried out using a PCR reaction specific for this strain [8]. The products of this reaction were analysed by electrophoresis on 2% agarose gels.

The data underwent least squares analysis using the GLM procedure [9]; the Pearson correlation coefficients between the parameters of the dry fermented sausages were calculated according to the

CORR procedure.

III. RESULTS AND DISCUSSION

The addition of the probiotic to the basic starter culture did not affect the course of the sausage fermentation (as pH and a_w) (Fig. 1 and Table 1). The pH levels can be interpreted according to data obtained in the microbiological analysis – little change in the number of lactic acid bacteria. Decreases in pH (groups II and III) on the third day of drying/ ripening have also been seen in another study [10].

The greatest weight loss was in group III (Table 1), due to the modified composition (i.e. the reduced fat content). Statistically significant lower weight losses were seen in group II, with the added probiotic and prebiotic, as compared to the standard, control, group I.

Group III was significantly different from the other two groups for fat, protein and ash content. Surprisingly, despite the greatest weight loss in this group, the highest water content was also seen, which can be attributed to the technological properties of the added emulsion (fat substitutes).

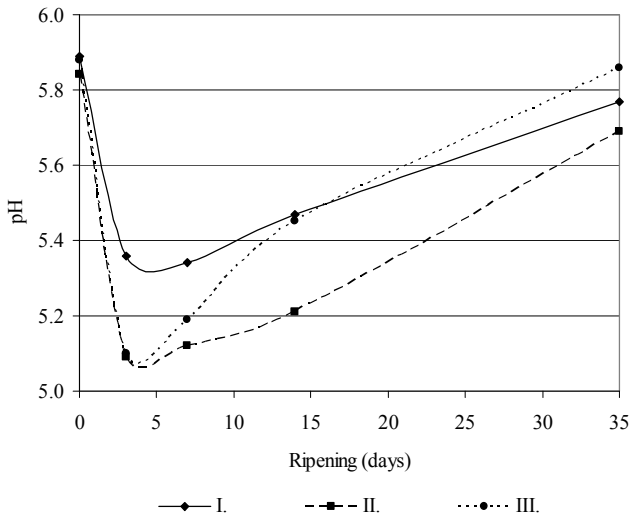

Figure 1. Effects of probiotic/ prebiotic addition and ripening period on pH of the dry fermented sausages. Groups I, II, III: see Methods.

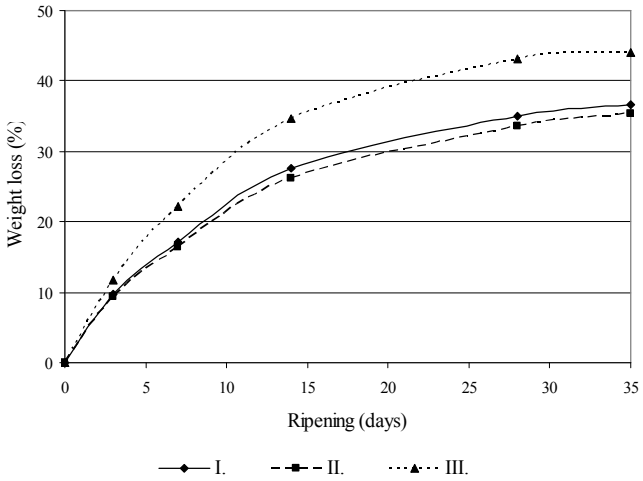

Figure 2. Effects of probiotic/ prebiotic addition and time of ripening on weight loss (%) of the dry fermented sausages. Groups I, II, III: see Methods.

The maximum calculated energy value of these dry fermented sausages was seen for the control, commercial meat starter culture alone, group I: 1,798 kJ/100 g. Similarly, as expected, the minimum calculated energy value was for group III, with the changed composition, as a reduced fat content: 1,568 kJ/100 g. This was 23% lower than for group I. Finally, the difference in the calculated energy value between groups I and II was minimal, with group II at 1,779 kJ/100 g.

The initial inoculation of the probiotic *L. paracasei* subsp. *paracasei* at a concentration of 1.25×10^7 CFU/g with the basic commercial starter culture (group II, Table 1) did not have any negative effects on the sensory properties of the products. In contrast, the products had better shapes, with a less wrinkled surface, and the aroma and the overall impression of these products were evaluated as better than those for the products with the standard, control, recipe; however, these differences between the groups were generally not statistically significant.

Table 1. Technological, chemical, instrumental, sensory and microbiological parameters of the dry fermented sausages with respect to probiotic and prebiotic addition, and reduced fat content in the sausage batter.

Parameter	Group I (LSM)	Group II (LSM)	Group III (LSM)	SEM	Sign.
a_w	0.879[a]	0.892[a]	0.884[a]	0.007	Ns
pH	5.77[a]	5.69[a]	5.86[a]	0.02	Ns
Weight loss (%)	36.7[b]	35.4[c]	44.1[a]	0.2	***
Chemical composition (%)					
Water	34.51[a]	34.97[a]	36.34[a]	0.67	Ns
Fat	34.74[a]	34.03[a]	27.51[b]	0.83	***
Protein	26.12[b]	26.01[b]	28.53[a]	0.42	**
Ash	5.39[b]	5.01[c]	6.00[a]	0.07	**
NaCl	3.59[a]	3.52[a]	3.72[a]	0.10	Ns
Inulin	0.00[b]	1.59[a]	1.62[a]	0.02	***
Instrumental measurement of texture (N)					
Kramer	141.6[b]	138.7[b]	251.3[a]	6.7	***
Volodkevich	4.15[b]	5.20[b]	7.31[a]	0.6	**
Sensory properties (scores)					
Shape (1-7)	4.7[ab]	5.0[a]	4.5[b]	0.1	**
Sausage surface (1-7)	3.3[b]	2.8[c]	4.0[a]	0.1	***
Colour intensity (1-7)	4.8[a]	4.8[a]	5.0[a]	0.1	Ns
Slice springiness (1-4-7)	3.3[b]	3.4[b]	3.9[a]	0.1	***
Texture (1-4-7)	3.3[b]	3.4[b]	3.9[a]	0.1	***
Smell (1-7)	5.2[a]	5.2[a]	5.3[a]	0.1	Ns
Flavour (1-7)	4.8[a]	5.0[a]	5.0[a]	0.1	Ns
Saltiness (1-4-7)	4.1[b]	4.1[b]	4.3[a]	0.1	*
Overall impression (1-7)	4.8[a]	5.0[a]	5.0[a]	0.1	Ns
Microbiological analysis (log CFU/g)					
TCC	8.13[a]	7.72[b]	7.88[c]	0.044	***
LAB	7.80[a]	7.60[b]	7.74[a]	0.046	*
Lb. paracasei	0.00[c]	7.37[b]	7.47[a]	0.026	***
TCC (2 months)	7.89[a]	7.48[c]	7.76[b]	0.044	***
LAB (2 months)	7.15[b]	7.23[b]	7.50[a]	0.067	**
Lb. paracasei (2 month)	0.00[b]	7.09[a]	7.18[a]	0.084	***

LSM, least-square means
SEM, standard error of the mean
Şign., levels of significance: statistically significant $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$; highly statistically significant: $^{***}P \leq 0.001$; statistically not significant: Ns – $P > 0.05$
Means with a different superscript within rows (a, b, c) differ significantly ($P \leq 0.05$)
TCC, total cell count
LAB, lactic acid bacteria
2 months, after the two-month storage period.

There were no negative effects of the addition of the prebiotic inulin at 1% (group II) on the sensory quality parameters, except on the sausage shape. A study has also shown that addition of powdered inulin (7.5%, 12.5%) is an excellent replacement for fat (25% reduced) in dry fermented sausages because it provides a softer texture and a tenderness, springiness and adhesiveness that is very similar to that of conventional sausages [11]. Products of group III have significantly better slice springiness, almost optimal texture, and a slightly higher score for colour intensity (not significant). These findings are in agreement with the literature data [12]. From the present study, it can be concluded that these differences were probably a consequence of the changed sausage composition, and not of the addition of the probiotic and prebiotic.

Texture was evaluated by scoring on a scale of 1-4-7. Four points were considered optimal, with 4.5 or more indicating extra firmness, and 3.5 or less indicated a more tender texture. Sausages from group III showed an almost optimal texture, while the control group I and group II were significantly more tender. Sensory evaluation was confirmed by instrumental analysis. There was significant, positive and tight correlation between the sensory evaluations of both slice springiness and texture and the instrumental

measurement with the Volodkevich bite jaw (R = 0.96; $P \leq 0.001$ and R = 0.95; $P \leq 0.001$, respectively). The correlation between weight loss and instrumental measurements with the Kramer shear cell was also statistically significant (R = 0.94; $P \leq 0.001$).

Microbiological analyses were carried out on the final products at the end of ripening and after two months of storage (packed products) at a temperature of 8 ° C to 10 ° C. According to the results obtained (Table 1), there were statistically significant differences between the groups for total cell counts, both at the end of ripening and after the two-month storage. Statistically significant differences in the number of lactic acid bacteria were also seen. The highest counts of lactobacilli at the end of ripening were in the products made with the standard recipe (group I), and after the two-month storage in the group with added probiotic and prebiotic, and modified composition (group III).

A statistically significant difference in the number of *L. paracasei* between groups II and III was seen at the end of ripening, while after the two-month storage this difference was no longer significant. A total of 12.5 g of probiotic culture (10^{11} CFU/g strain *L. paracasei* subsp. *Paracasei*) was added to 100 kg of sausage batter. So, the initial inoculation with the probiotic *L. paracasei* subsp. *paracasei* was at 1.25×10^7 CFU/g of batter. In final products, the number of *L. paracasei* in both of these groups was higher than 10^7 CFU/g, although after the two-month storage this was slightly lowered. Accordingly, these results indicate that these dry fermented sausages are an appropriate medium for this probiotic, as the *L. paracasei* subsp. *paracasei* survived throughout the fermentation, ripening and two-month storage at 8 °C to 10 °C, and was present in the final products at the predicted level of 10^7 CFU/g or more.

Validation of the survival of *L. paracasei* isolated from dry fermented sausages was also carried out by analysis of the DNA amplification products by PCR. In the example in Figure 3, lanes 13 and 26 were a 100-bp-size DNA standard (Fermentas), and lanes 24 and 25 were a positive control (*L. paracasei* DSM 5622 and *L. paracasei* FD-DVS L. casei-01 nutrish®). Lanes 1 to 12 and 14 to 20 show the DNA from randomly selected colonies from LAMVAB medium that were typical in appearance to *L. paracasei* (small white colonies); lanes 21 to 23 were DNA from three colonies that were identical in size to those of *L. paracasei*, but that differed in appearance. The arrows indicate the position of the expected size of 290 bp that is characteristic of *L. paracasei*. Since the translucent colonies did not show this characteristic amplification, they were not included in the *L. paracasei* count. The results show that most of the colonies that had an appearance similar to that expected for *L. paracasei* did indeed belong to this strain, and were included in the count.

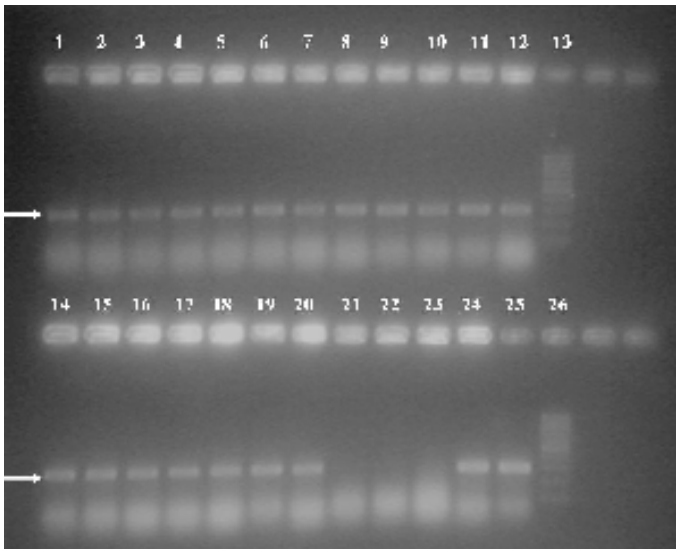

Figure 3. Amplification products obtained from the PCR reaction specific for the *L. paracasei* strain, as representative data from electrophoresis on a 2% agarose gel.

IV. CONCLUSION

We have succeeded here in producing dry fermented sausages with functional properties that meet modern nutritional requirements for products with a low fat content or a lower energy value. These products also have convenient sensory properties, and at the same time they can be classified as safe and healthy food.

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