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METTWURST FERMENTED BY PROBIOTIC *L. CASEI* AND *L. ACIDOPHILUS* STRAINS

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Abstract

The ability of two probiotic strains, L. casei LC-01 (Sacco, Italy) and L. acidophilus La-5 (Sacco, Italy) to produce fermented Mettwurst were studied. Probiotic cultures were applied of such amounts to reach the level of minimum 10^6/g of the end-product. Each probiotic strain was used in combination with commercial starter culture (Pediococcus pentosaceus AS-3/100; Almi, Austria) commonly used for Mettwurst production. Control sausages were made using only the starter strain.

Bacterological media used for enumeration of bacteria were MRS for enumeration of both starter and probiotic bacteria, MRS–moxalactam (112 mg/l) for separately enumeration of L. casei and MRS–clindamycin (0,5 mg/l) for separately enumeration of L. acidophilus, respectively. In both probiotic batches, the numbers of the probiotics, L. casei and L. acidophilus, respectively, reached the level of 10^6 during fermentation and stayed at relatively constant level during the whole storage period (21 days) at 4 °C.

The sensory profiles of the experimental Mettwurst sausages produced by these probiotics were evaluated. Determination of general appearance, flavour and taste, specially of bitter, sweet, sour, metal and salt taste were evaluated. The biggest changes in sensory quality between probiotic and control sausages were in salt and sour taste. Probiotic cultures L. casei LC-01 and L. acidophilus La-5 are suitable for use in manufacture of fermented Mettwurst sausages.

Key words: *probiotic, L. casei, L. acidophilus, Mettwurst, fermentation*

1. INTRODUCTION

The sausages of the type Teewurst and Mettwurst are risk-products from the microbiological point of view because they are produced from raw meat and fat and are only briefly fermented (Rodel and Scheuer, 2001; Buckenhuskes and Gehring, 2000). During sausages fermentation, complex biochemical and physical reactions take place that result in a significant change in the initial characteristic (Tjener *et al.*, 2003; Swetwiwathana *et al.*, 2008). Some probiotic cultures we can use as nontraditional meat starter cultures in production of ferment meat products. They ensure the rapid onset of fermentation and discourage growth of undesirable bacteria by production of acids and reduction pH (Pidcock *et al.*, 2002) and changes are in sensory evaluation too (Casaburi *et al.*, 2007; Garriga *et al.*, 1996). A probiotic is a culture of living microorganisms – mainly lactic acid bacteria (LAB), e.g. *L. casei*, *L. acidophilus* and bifidobacteria – which have a beneficial effect on a health of the host when ingested in certain amounts (FAO/WHO, 2002; Fuller, 2000; Leahy *et al.*, 2005; Huang and Adams, 2004). Oral consumption of probiotics is suggested to have a positive effect on the maintenance of the intestinal microflora and the colonisation resistance against pathogens as well as to alleviate symptoms of lactose resistance and promote beneficial immune response (Erkkilä *et al.*, 2001). The suggested concentration for probiotic bacteria is in range 10^6 – 10^7 cfu/g of the product (Robinson, 1987, Ruiz-Movano *et al.*, 2008). The reliable determination of viability of bacteria in probiotic food is important (Lahtinen *et al.*, 2006). The growth of LAB in fermented sausages depends

on their ability to tolerate the salty concentration, pH values and water activity of these products (Martín *et al.*, 2007). Traditional LAB are counted in MRS Agar, but this is not selective medium for enumeration of probiotic microorganisms when non-probiotic starter cultures are present (De Carvalho Lima *et al.*, 2009). Andersen (1998) using mixture starter culture Bactoferm T-SPX (Ch. Hansen) and probiotic culture of *Lactobacillus casei* LC-01 to fermented meat products.

The aim of this study was to compare growth capacity of probiotic microorganisms *L. casei* and *L. acidophilus* in fermented sausage "Mettwurst" and compare their influence on microbiological profile, pH value, and sensory quality of these products. The other aim was to employ species-specific PCR methods for DNA confirmation of presumptive *L. casei* and *L. acidophilus* colonies.

2. EXPERIMENTAL STUDY

2.1 Materials

2.1.1 Strains

The pure lyophilized commercial starter culture of *P. pentosaceus* AS-3/100 (Almi, Austria) and two probiotic cultures *L. casei* LC-01 (Sacco, Italy) and *L. acidophilus* La-5 (Sacco, Italy) were used to produce fermented "Metwurst". Each probiotic strain was used in combination with starter culture *P. pentosaceus* which is commonly used for Mettwurst production. Control sausages were made using only the starter strain. The lyophilized cultures were maintained under -20 °C until used.

2.1.2 Mettwurst sausages

The sausage "Mettwurst" contained pork and back fat (50 %), beef (45 %), sucrose (0,1 %), nitrite salt (2,46 %), 0,43 % of ground black pepper, paprika and garlic, starter culture *P. pentosaceus* (0,25 %), probiotics *L. casei* (0,02 %) or *L. acidophilus* (0,02 %). The starter culture and probiotics were added to achieve 10^6 at minimum cfu/g in sausage mixture. The final sausage mixtures were stuffed into 4,3 cm diameter casings (Nalo Krans N43) to make up individual pieces weighing 1 kg and smoked by cold smoke 20 °C intermittently for 2 days. The samples of "Mettwurst" were stored at 4 °C for 21 days.

At each sampling period (days 0, 7, 14 and 21) samples of „Mettwurst" were taken for microbiological analysis, determination of pH and sensory evaluation.

2.2 Methods

2.2.1 pH value

The pH value was measured directly from the sausage (WTW Microprocessor pH 95; Wissenschaftl.-Techn.) as a mean value of three measurements immediately after the production (0 day) and at 7, 14 and 21 days of ripening.

2.2.2 Microbiological analysis

Ten grams of "Mettwurst" were diluted in 90 ml of sterile Ringer's solution (Merck, Germany). Sausages were examined immediately after production (0 day) and at 7, 14 and 21 days of ripening. Mesophilic LAB were enumerated on MRS agar (deMan, Rogosa and Sharp; Noack, France) in anaerobic conditions for 48 h at 37 °C. The numbers of inoculated probiotic bacteria were determined by using MRS–moxalactam (112 mg/l) for separately enumeration of *L. casei* and using MRS–clindamycin (0,5 mg/l) for separately enumeration of *L. acidophilus*, respectively. The incubation was carried out in anaerobic conditions for 48 h at 37 °C.

2.2.3 Sensory evaluation

The samples of "Mettwurst" were submitted to sensory evaluation to ascertain whether differences existed between control sample (*Pediococcus pentosaceus* AS-3/100) and samples inoculated with probiotic cultures (*L. casei* LC-01, *L. acidophilus* La-5). The sensory evaluation was performed immediately after the production (0 day) and at 7, 14 and 21 days of ripening by 10 trained members in sensory laboratory equiped according to ISO 8589. Determination of general appearance, flavour and taste, specially of bitter, sweet, sour, metal and salt taste were evaluated, using a continuous unstructurated scale (10 cm) without references. Panel data were collected by means of Unistat Ltd. software and analyzed by means of Tukey's test ($P<0,05$ and $P<0,010$) for multiple comparisons.

3. RESULTS AND DISCUSSION

There is a world wide trend toward new probiotic food and Mettwurst may be another application area. The development of new probiotic meat products presupposes the application of probiotic bacteria with at minimum resistance to sodium chloride and growth and activity during fermentation and ripening of meat products. Standards requiring a minimum of 10^6 cfu/g of probiotic cells in fermented milk products have been introduced by several food organizations worldwide (IDF, 1992; Shah, 2000; Bibiloni *et al.*, 2001). For meat products, no standards still do not exist, but to provide health benefits, the same criterion as for milk products (10^6 cfu/g) is required. It becomes important therefore, that suitable techniques are developing to accurately enumerate the counts of probiotic bacteria, in the presence of starter cultures, in meat products.

3.1 pH value and count of microorganisms

At 7 days of ripening the pH values of the Meetwurst were 4,72 (control batch), 4,42 (bath with probiotic *L. casei*) and 4,39 (bath with probiotic *L. acidophilus*). At the end of ripening time (21 days) the pH of the sausages fermented by commercial starter culture only was 4,66, while the pH of the sausages fermented by starter and probiotic *L. casei* was 4,28 and by starter and probiotic *L. acidophilus* was 4,20. Development in pH value during minimal endurance time (21 days) shows Fig. 1. Fig. 2 shows counts of lactic acid bacteria (LAB) enumerated on MRS agar during 21 days of Meetwursts ripening. LAB grew slowly during first days of fermentation. Between 7 to 14 days of ripening the number of LAB in control batch and in batch with LA-5 grew rapidly. Decline of LAB in batches with Lc-01 and with La-5 were at the last week to the end of fermentation. LAB in control batch AS-3/100 grew rapidly.

LAB decreased the pH of the sausages under pH 5,0 during first days of fermentation, which acted as a hurdle for several Gram-negative bacterial species. Also the growth of spores, mainly of the genus *Bacillus* and *Clostridium*, which could come from meat or spices, was controlled by low pH, as described in Nordal and Gudding (1975).

The bathes with *L. acidophilus* and *L. casei* were faster acidificated than the control. *L. acidophilus* lowered the pH more than *L. casei.* The results show that using probiotic cultures can decrease pH of Mettwursts better than the starter culture *P. pentosaceus*.

The presence of life probiotic Lc-01 and La-5 cells documents the Fig. 3. Both, *L. casei* and *L. acidophilus*, were detected at the concentrations of 10^6 CFU/g during the whole ripening period. *L. acidophilus* had faster growth rate than *L. casei.*

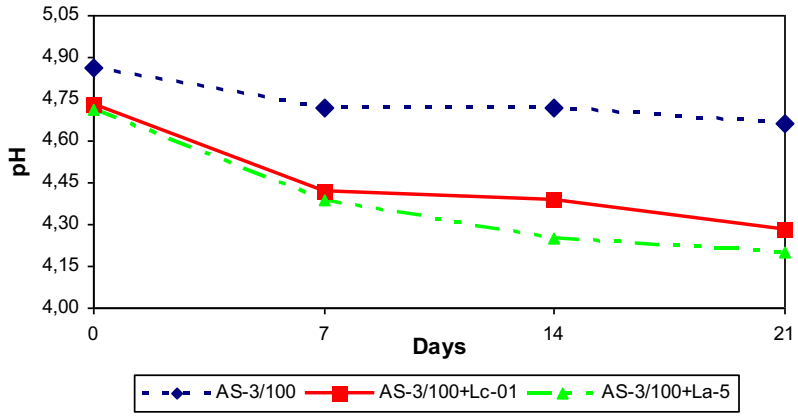


Fig. 1 Development of pH in fermented sausages "Mettwurst" with starter culture AS-3/100 (♦) and probiotic cultures AS-3/100+Lc-01 (■) and AS-3/100+La-5 (▲)

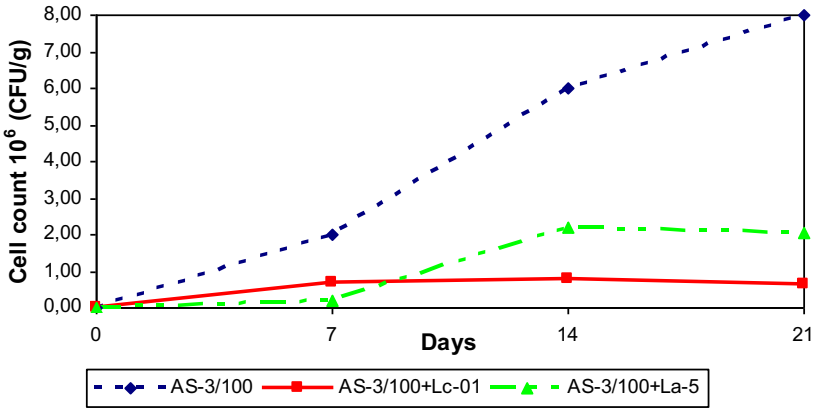


Fig. 2 Development of starter AS-3/100 (♦) and probiotic strains AS-3/100+Lc-01 (■) and AS-3/100+La-5 (▲) in fermented sausage "Mettwurst" during minimal endurance time of 21 days

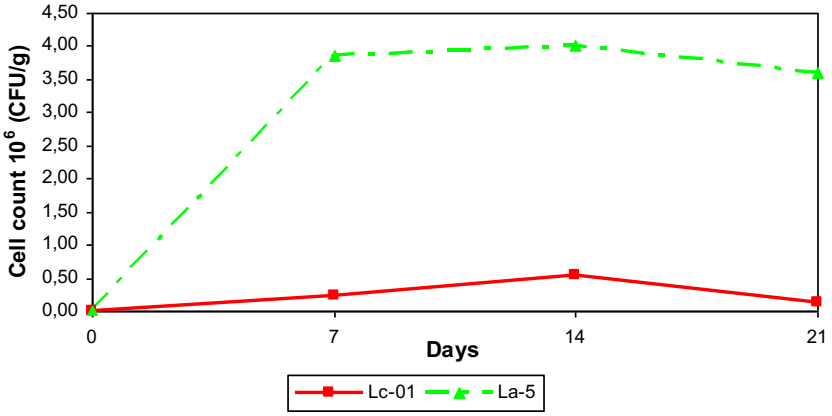


Fig. 3 Development of probiotic strains *Lc-01 (■), La-5 (▲) in fermented sausage "Mettwurst" during minimal endurance time 21 days*

Besides contributing to human health, probiotic fermented meats need to be of sufficient commercial value. Therefore, a primary requirement remains the sensory and technological aspects of the end product. Negative side effects on the overall quality of the obtained fermented meats can not be tolerated and evaluation is primordial (De Vuyst *et al.*, 2008).

The typical flavour of Meetwurst is a result of the interaction of numerous compounds resulting from the microbial and muscle enzyme metabolism of carbohydrates, proteins and lipids in combination with spices (Flores et al., 1997; Toldra, 1998). Starter cultures should bring and stabilize the desired sensory properties. Mostly, the bacteria of the genera *Lactobacillus* and *Pediococcus* are used in fermented meat production. When producing probiotic meat products, the sensory and technological aspects of the end-products have to be near the traditional products. Negative side effects on the overall quality of the obtained Mettwurst can not be tolerated and evaluation of such effects is primordial.

Fig. 3 shows the sensory profile of samples AS-3/100+Lc-01 and AS-3/100+La-5 compared with control sample AS-3/100. Determination of general appearance, flavour and taste, especially of bitter, sweet, sour, metal and salt taste were evaluated. The biggest changes were in descriptors salt and sour taste, pleasantness of taste, intensity of taste and intensity of odour. Samples with probiotic cultures were evaluated better than control sample all the time but sourness and saltiness were expressive. Immediately after the production (0 day) sensory evaluation was better at samples with probiotics especially in descriptors pleasantness of odour, pleasantness and intensity of taste. But the sourness, saltiness and metal taste were intensive in sample AS-3/100+La-01. After 14 days of fermentation descriptor of saltiness were the biggest in sample SA-3/100+Lc-01 the same as at 7 days of fermetation. Well-balance sensory evaluation was 14 days from processing. The descriptors of taste and smell were well-balanced at the all samples and high level of saltiness and sourness were inhibited. After 21 days (end of minimal endurance time) the biggest changes was in sample AS-3/100+Lc-01. This sample had the worst sensory evaluation in descriptors pleasantness of odour, intensity of odour and taste and the biggest amount of sour taste and pH value decline expresivelly than other samples. Whereas the sample AS-3/100+La-5 and control sample AS-3/100 were evaluated better but not as good as at 14 days. We can say that samples with probiotic microorganisms were better evaluted at the first past and sample with starter culture at second past of storage life. One of the reasons could be decline of pH value. For that reason we must entertain the shortening of minimal endurance time.

When applying the probiotic cultures Lc-01 and La-5 with a traditional starter culture, no negative impact on the sensory properties of the product was noticed. Panel data were analyzed by means of Turkey's test for multiple comparisons. There is no significant differences in parameters between samples ($P<0,05$ or $P<0,010$).

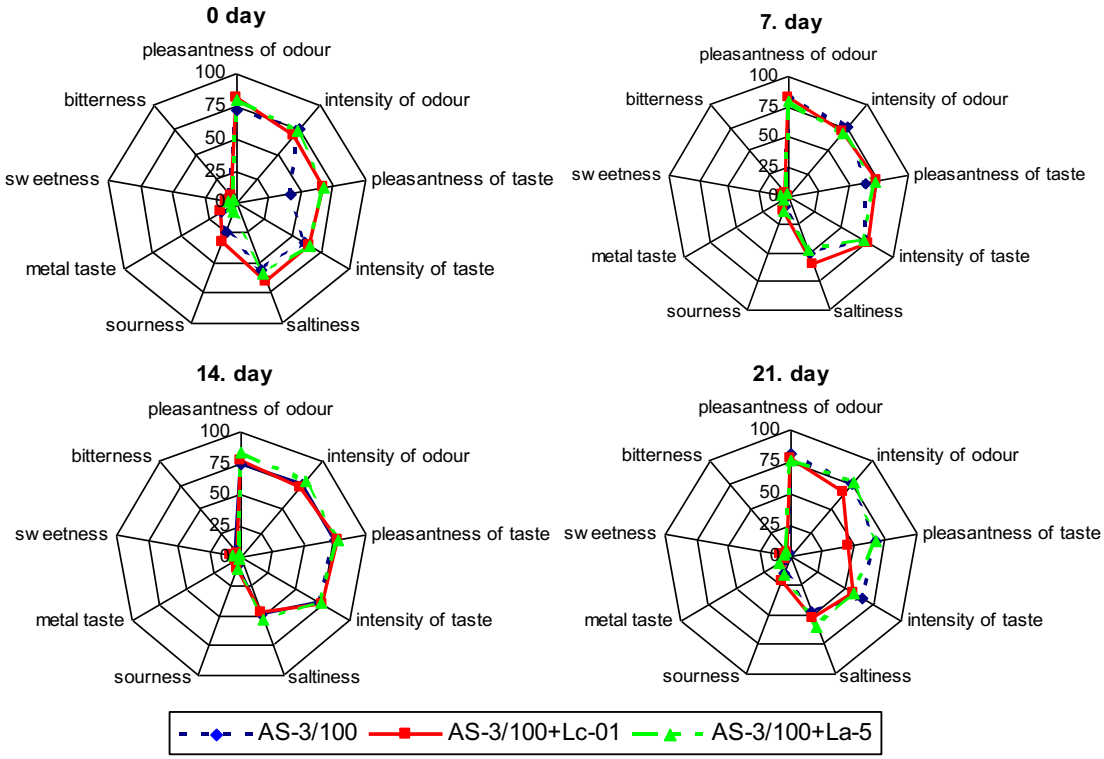


Fig. 4 Sensory profiles of control "Mettwurst" AS-3/100 (♦) compared with sensory profiles of inoculated sausages AS-3/100+Lc-01(■) and AS-3/100+La-5 (▲) during fermentation

4. CONCLUSIONS

Mettwursts are non-heated meat products which may be suitable carrier for probiotics into human gastrointestinal tract. The application of probiotic cultures in fermented Meetwurst in conjunction with low fat and low salt content might have health promoting effects and probiotics must ensure sensory quality of products.

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