THE SYNERGISTIC EFFECT OF STARTER CULTURES AND FREEZE-DRIED VEGETABLES ON THE FERMENTED SAUSAGES RIPENING PROCESS

R. Vinauskienė[1*], V. Eisinaitė[1], D. Leskauskaitė[1]

[1]Department of Food Technology, Kaunas University of Technology, Lithuania

Abstract - The fermented sausages were produced with the addition of 3 % lyophilized vegetables: celery, parsley or parsnip, fastening the fermentation process with microorgnisms: *Pediococcus pentosaceus, Staphylococcus xylosus, Staphylococcus carnosus*, which have nitric reducing properties. The 4 days long ripening process was evaluated by change of pH, water activity and amount of microorganisms in the sausages. The obtained results showed that all three lyophilized vegetables intensified the changes of a_w values, which reached 0.947-0.950 in the final products. Recorded more intensive reduction of pH value during ripening of sausages with added vegetables was observed because of the increase of total carbohydrates content caused by the addition of vegetables. Microbiological analysis showed that added vegetables did not promote the growth of the starter culture microorganisms, but inhibited the growth of coliform bacteria – their amount did not change in the period of 4 days. During the testing time, the amount of coliform bacteria in the control sample increased 10^3 times.

Key Words - Freeze-dried vegetables, Celery, Parsley and Parsnip, Starter culture, Fermented sausage, Ripening

I. INTRODUCTION

Looking for the possibilities to reduce the amount of synthetic food additives in meat products, it is necessary to ensure their functions by different technological means. For this purpose vegetable additives can be used as a good resource of fiber, carbohydrates and bioactive plant ingredients, which are needed to ensure the quality of fermented meat products. Celery, parsley and parsnip were chosen for this investigation, because they accumulate vitamins and preservatives – nitrates, forming the meat color and have strong antioxidative properties [1]. Their processing by lyophilization protects bioactive materials from the degradation. Starter cultures, chosen for this investigation have nitrate reducing properties. The aim of the study was to investigate the synergistic effect of starter cultures and freeze-dried vegetables powder on the ripening process of fermented sausages.

II. MATERIALS AND METHODS

Lyophilization of vegetables: chopped celery, parsley and parsnip roots were frozen at minus 18 ^oC temperature. Frozen vegetables were dried using a vacuum of 4.0 mbar, which at the end of drying was reduced to 0.50 mbar. Lyophilized vegetables were crushed to powder-like state.

Production of fermented sausages: sausages were made from 97 kg of ground pork ham with 3 kg lyophilized vegetables (parsnips, parsley or celery). 2.7 kg salt (NaCl), 0.2 kg pepper, 0.1 kg coriander, 0.5 kg dextrose and 0.16 kg starter culture mixture *(Pediococcus pentosaceus, Staphylococcus xylosus Staphylococcus carnosus)* were also added to all sausages. As control the sausages without additives and with starter culture addition only were used. Sausages were ripened in an universal thermal camber Bastramat 850 C-UF (Bastra, Germany) for 4 days. During ripening the temperature was reduced from 24 ^oC to 19 ^oC, and relative humidity from 92 % to 84 %.

The ripening process was evaluated by changes of pH, water activity and total count of microorganisms. The water activity was measured by a Moisture analyzer (Metler Toledo, Switzerland) pH was measured using WTW 3110 pH-meter (Germany). Colonia forming units (CFU) of mesophilic lactic bacteria, *staphylococcus* and coliforms was estimated cony-count standard methods [2, 3, 4].

III. RESULTS AND DISCUSSION

During meat fermentation process, the homofermentic lactic acid bacteria, naturally presenting in the meat, or added on purpose, are fermenting the carbohydrates and producing lactic acid. That is why the pH level is decreasing and can reach pH 4.6 – 5.3 in the final product. It is important to ensure, that the decrease of pH will be as fast as possible; if the required balance between pH level and decrease of humidity is not reached, bacterial spoilage of meat can occur during fermentation [5]. In appropriate conditions (temperature, relative humidity, sugars content), the starter cultures inhibit the fermentation process and the pH value, ensuring the product safety is reached faster [6].

In this study it was established that after 4 days of ripening of sausages made with lyophilized vegetables and added starter cultures, the pH value varied in the range from 5.66 – 5.73 to 4.93 - 5.08. As it is seen in Figure 1, the pH value was mostly influenced by the addition of parsnip (4.93). In the control sausage made with starter cultures, but without vegetables, the pH value was decreasing slower during all the fermentation process and after 4 days pH was only 5.27. That can be explained by the fact, that in the sausages with vegetables, the starter cultures used carbohydrates from the vegetables as nutritional substances, therefore the pH lowered faster. The most different changes of pH were recorded for the control sausage without vegetable addition; during fermentation of this sausage the pH value changed only from 5.68 to 5.62.

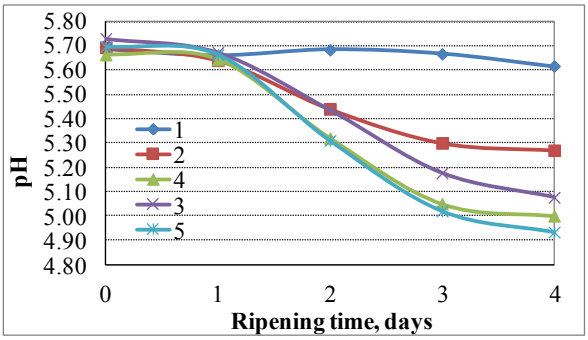

Figure 1. Changes of pH during ripening of sausages with different additives of vegetables: 1- Control; 2 - Control with starter culture addition; 3 - Sausage with parsley and starter culture addition; 4 - Sausage with celery and starter culture addition; 5 - Sausage with parsnip and starter culture addition;

Similar results were obtained by investigating the ripening process of Turkish beef sausages. In the sausages, ripened for 4 days at 25 ^{o}C temperature and 85 – 90 % relative air humidity with 0,5 % sucrose and addition of *Staphylococcus xylosus* and *Pediococcus pentosaceus* starter cultures, the pH value decreased from 5,73 to 4,90 [9].

The changes of water activity, which describes the drying of sausages during ripening, has been quite uneven: after the first day of ripening a_w slightly increased in all samples, and in the third day – it started decreasing. It should be noted that the smallest values of the water activity after the fermentation process were obtained in sausages with celery addition (0.947 – 0.950), and the highest – in the control sausages, made with starter culture (0.955 – 0.961) (Figure 2.) In stable environmental conditions most of the microorganisms stop to reproduce, when a_w is lower than 0.91, with the exception of *Staphylococcus* and fungi, so the ripening of the products was carried on [8]. The decreasing pH and water activity values correlated, ensuring the microbiological safety of the products.

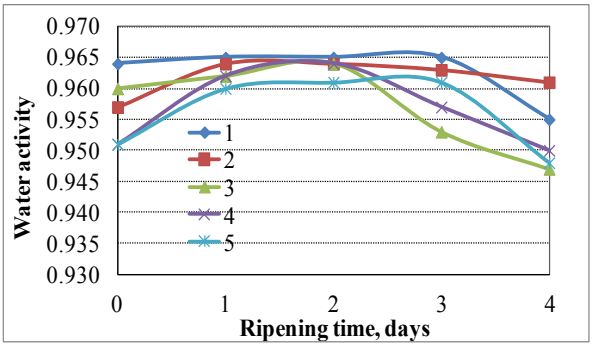

Figure 2. Changes of water activity during ripening of sausages with different additives of vegetables:
1- Control; 2 - Control with starter culture addition;
3 - Sausage with parsley and starter culture addition;
4 - Sausage with celery and starter culture addition;
5 - Sausage with parsnip and. starter culture addition;

The microbiological analysis had shown that additives of vegetables did not inhibit the growth of starter cultures, while the growth of coliform bacteria by these additives was suppressed after 4 days of ripening.

It was established that the log (10) number of total count of *Pediococcus pentosaceus* was 2 time

higher in sausage with starter culture in comparison with the control sausage without starter culture at the beginning of ripening and varied from $1.9x10^6$ to $3.0x10^6$ CFU/g depending on the sort of added vegetables (Figure 3.). An increase of *Pediococcus pentosaceus* in all sausages correlated with the decrease of pH and in the end of ripening the number of bacteria reached $1.9x10^7$ and $1.1x10^8$ – $3.5x10^8$ CFU/g in the control sausages and sausages made with the addition of lyophilised vegetables, respectively. In accordance with other authors, the proper fermentation of sausages is characterized by increasing number of lactic acid bacteria from 10^3–10^5 to 10^6–10^9 CFU/g during the first three days of fermentation or 1 – 14 days of ripening. Usually these bacteria are the predominant microflora throughout all process [9].

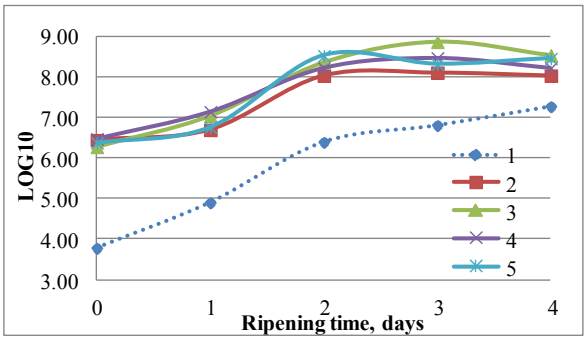

Figure 3. Changes of CFU of *Pediococcus pentosaceus* during ripening of sausages with different additives of vegetables: 1 - Control; 2 - Control with starter culture addition; 3 - Sausage with parsley and starter culture addition; 4 - Sausage with celery and starter culture addition; 5 - Sausage with parsnip and. starter culture addition;

Results of microbiological analysis show that the number of nitrate reducting *Staphylococcus carnosus* and *Staphylococcus xylosus* did not increase in the sausages made with lyophilised vegetables after the first day of ripening. However the growth of bacteria was observed after the second day of ripening and the number of bacteria reached $1.2x10^7$ – $4.6x10^7$ CFU/g at the end of the process (Figure 4.).

Similar results were observed in the control sausages made with the addition of starter cultures only. According to the literature data, the growth of *coccus* is reduced by growth of lactic acid bacteria as they induce the decrease pH of medium. [9,10]. The analogous results were observed in the control sausages made without addition of starter cultures, however, in this case the number of bacteria increased from $9.0x10^3$ to $4.5x10^5$ CFU/g after the first day.

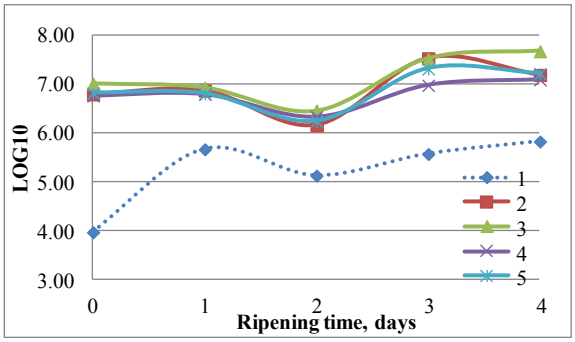

Figure 4. Changes of CFU of *Staphylococcus xylosus* and *Staphylococcus carnosus* during ripening of sausages with different additives of vegetables:
1 - Control; 2 - Control with starter culture addition;
3 - Sausage with parsley and starter culture addition;
4 - Sausage with celery and starter culture addition;
5 - Sausage with parsnip and. starter culture addition;

Significant differences in coliform bacteria number was observed between control sausages made without starter cultures and other sausages in the start and end of the ripening process (Figure 5.). The log (10) number of coliform bacteria increased from $4.0x10^1$ to $1.0x10^4$ CFU/g (i.e. almost 4 times) in control sausages, while formation of lactic acid in other products reduced their pH and increased the stability of the products.

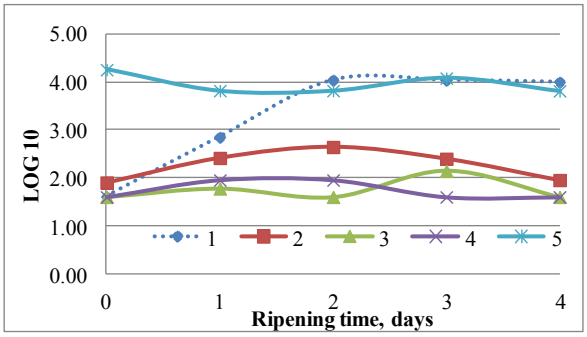

Figure 5. Changes of CFU of coliforms during ripening of sausages with different additives of vegetables:
1 - Control; 2 - Control with starter culture addition;
3 - Sausage with parsley and starter culture addition;
4 - Sausage with celery and starter culture addition;
5 - Sausage with parsnip and. starter culture addition

Furthermore, the growth of spoilage bacteria is reduced by formation of organic acid, diacetyl and bacteriocins during fermentation process [11].
The higher values of pH and water activity (a_w) made favorable environmental conditions for the growth of coliform bacteria in control sausages. The lowest coliform bacteria content ($4.0x10^1$ CFU/g) was recorded in the sausages made with addition of lyophilised parsley and celery before and after ripening of sausages. The higher number of coliform bacteria recorded in the samples made with parsnip was caused by the contamination of lyophilised parsnip by coliform bacteria – $9.8x10^4$ CFU/g, however the number of coliform bacteria decreased from $1.8x10^4$ to $6.5x10^3$ CFU/g in the sausages made with the addition of this lyophilised vegetable.

IV. CONCLUSION

Freeze-dried celery, parsley and parsnip influenced the ripening process of fermented sausages:

- Freeze dried vegetables intensified the changes of a_w values;
- The pH level decreased faster, because of the carbohydrates presented in the vegetables;
- The added vegetables did not promote the growth of starter cultures, but inhibited the growth of coliform bacteria.

ACKNOWLEDGEMENTS

This research was funded by a grant (No. SVE-07/2011) from the Research Council of Lithuania.

REFERENCES

1. Yang Yao, Wei Sang, Mengjie Zhou & Guixing Ren. (2010). Phenolic Composition and Antioxidant Activities of 11 Celery Cultivars. Journal of Food Science Vol.75: c 9-c 13.
2. LST ISO 15214:2009. Microbiology of food and animal feeding stuffs - Horizontal method for the emumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 °C (ISO 15214:1998, identical.)
3. LST EN ISO 6888-1:2000. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase -positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium (ISO 6888-1:1999+Amd 1:2003).
4. LST ISO 4832:2006. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique (identical ISO 4832:2006).
5. Cerveny J., Meyer D. J. & Hall P. A., Microbiological Spoilage of meat and Poultry Products. (2009) In. Sperber W. H., Doyle M. P. Compendium of the Microbiological Spoilage of Foods and Beverages. (pp 69-86) Springer New York.
6. Fidel Toldrá (2007). Handbook of fermented meat and poultry, Blackwell Publishing.
7. Ůlků Dalmıs & Ayla Soyer. (2008) Effect of processing methods and starter culture (*Staphylococcus xylosus and Pediococcus pentosaceus*) on proteolytic changes in Turkish sausages (sucuk) during ripening and storage. Meat Science 80: 345-354.
8. Marianski, S. & Marianski A. (2008). Art of Making Fermented Sausages. Bookmagic, LLC.
9. Hui Y. H. (2012) Handbook of meat and meat processing, second edition, CRC press.
10. Pelle Thonning Olesen, Louise H. Stahnke (2004) The influence of environmental parameters on the catabolism of branched-chain amino acids by *Staphylococcus xylosus* and *Staphylococcus carnosus*, Food Microbiology, 21: 43-50
11. Simonova M., Strompfova V., Miroslava Marcinkova M., Laukova A., Vesterlund S., Moratalla M. L., Bover-Cid S. & Vidal-Carou C. (2006). Characterization of *Staphylococcus xylosus* and *Staphylococcus carnosus* isolated from Slovak meat products, Meat Science 73: 559-564.