EVALUATION OF POSSIBILITY TO USE *STAPHYLOCOCCUS CARNOSUS* ATCC 51365 BACTERIAL STRAIN IN NITRITE CURING PROCESS OF MEAT

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Abstract – The aim of the work was the application of the strain of denitrifying bacteria *Staphylococcus carnosus* ATCC 51365 in order to improve effectiveness of the nitrite meat curing process and examination of its influence on the selected quality features of the model meat products subject to heat treatment. The study was performed on the model forcemeat cured with sodium nitrite subject to heat processing. The original number of bacteria *S. carnosus* ATCC 51365 in the forcemeat was 10^7 CFU/g. The conducted study proved that the application of *S. carnosus* ATCC 51365 for nitrite curing of meat improves the effectiveness of the process and has a positive effect on the colour of the meat products subject to heat processing. The enrichment of the natural meat microflora with the *S. carnosus* ATCC 51365 bacteria strain contributed to the lowering of the level of nitrates in the model product subject to heat processing, as compared to the control variant after production and nitrates and nitrites after the 8-week storing period.

Key Words – meat products, curing, denitrifying bacteria , quality

I. INTRODUCTION

The basic reaction in the curing process, in result of which the characteristic colour of meat appears, is the formation of nitrosylmyoglobin. One of its formation stages are the reactions leading to the formation of nitric oxide from the added nitrites. During the curing nitrites take part in many competitive reactions in the meat [1]. Cassens et al. [2, 3] proved that 1 - 10% of nitrites added to meat transformed into nitrates. Some researchers indicate now that the quantity of nitrite oxidising during meat curing is higher and amounts to 10 ÷ 40% [1]. It is not finally clear in result of which chemical reactions significant quantities of nitrates are sometimes formed in the products curing with nitrites. It is known, however, that the total balance of nitrites added to meat during curing may be different and depends on many factors, i.e. biochemical properties of particular muscles, conditions of the technological process applied, meat microflora or application of substances supporting this process [4, 2, 3, 1]. Various bacteria strains may form meat microflora, including denitrifying bacteria that penetrate into meat during the slaughter of animals and partition of carcasses [5, 6]. The improvement of the hygiene level in the production halls as well as low temperature applicable there have a positive effect on the health quality of raw material but, simultaneously, they limit the appearance and development of the useful microflora in meat. The excessive "sterility" of the meat raw material and thus potentially different biochemical properties of forcemeat may contribute to the change of the process of chemical reactions in meat during curing. This situation occurs, in particular, in case of products cured with the use of nitrites and subject to heat treatment, since their production process is relatively short and it might not be sufficient for effective action of the desired meat microflora. One may presume that the limitation of the available nitrite during meat curing caused by its oxidising to nitrate may have a significant influence on process effectiveness and colour stability of the meat product subject to heat treatment.

The aim of the work was the assessment of the application of the strain of denitrifying bacteria *Staphylococcus carnosus* ATCC 51365 to the

improvement of effectiveness of the nitrite meat curing process and examination of its influence on the selected quality features of the model meat products subject to heat treatment.

II. MATERIALS AND METHODS

The study was performed on a model, finely ground meat forcemeat cured with sodium nitrite (100 mg/kg) in a can. The denitrifying bacteria strain *Staphylococcus carnosus* ATCC 51365 was applied, isolated from dried sausage. The original number of bacteria *S. carnosus* ATCC 51365 in the forcemeat was 10^7 CFU/g. The control variant did not contain added bacteria strain. The forcemeat was subject to heat treatment in stages in such a way that the temperature in the centre of the forcemeat was at the level of 20, 40, 45°C for two hours and then up to getting 70°C inside. The study was made in five parallel repetitions. The samples for examination were taken after the finished heat treatment and cooling the product and after 3 and 8 weeks of storing in the temp. 4°C. In the samples the following values were defined:

- contents of nitrates and nitrites [7],
- total number of oxygen micro-organisms (TSA substrate), using plate method,
- pH [8] and redox potential (Metter Delta 350 apparatus with InLab Redox Pro electrode),
- contents of nitrosylpigments with Horsney method [9],
- colour parameters in the system CIE L*a*b* (Minolta CR-300).

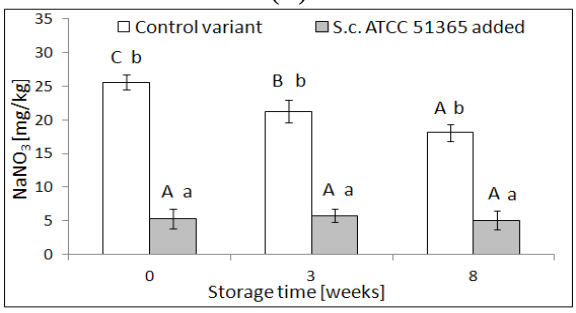
For the statistical analysis of results the Statgraphics Plus 4.1 program was applied. The single-factor variance analysis and the significance of differences between the average values was analysed with Fisher test. All the tests were tested at the significance level p=0.05.

III. RESULTS AND DISCUSSION

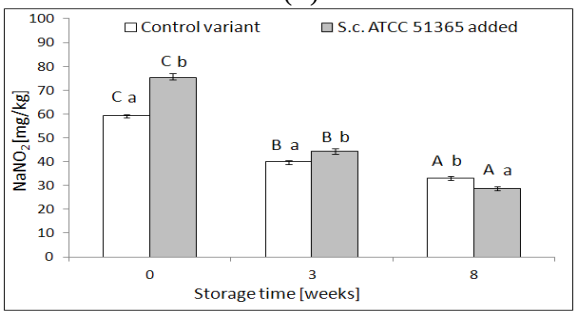
The performed studies proved that the enrichment of the natural meat microflora with the denitrifying bacteria strain significantly lowered the content of nitrates in the finished product. It was stated that in case of a control variant, about 25% of the nitrite added to meat had been oxidised to nitrate. In the sample with the applied strain *S. carnosus* ATCC 51365 the quantity of the resulting nitrate was nearly five times lower (Fig. 1).

Fig. 1 Nitrate (A) and Nitrite (B) content in the model products during storage

(A)



(B)

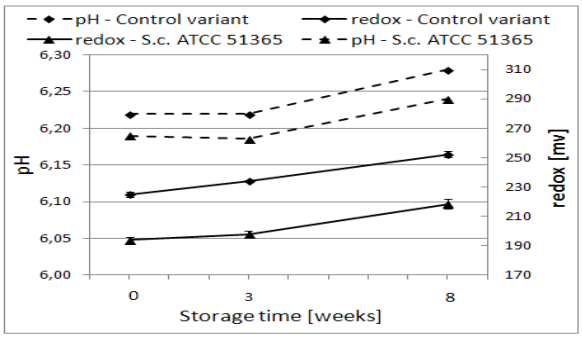


Explanatory notes: Values plotted on the graph are $\overline{x}$ ±SD. Mean values by different letters statistically significantly ($p \leq 0.05$)

The stated result was probably connected with the activity of the nitrate reductase enzyme and use of nitrates in the process of the anaerobic respiration of denitrifying bacteria.

It was determined that the content of nitrites decreased in both experimental samples during their storing ($p \leq 0.05$). The dynamics of those changes was bigger for the variant with the bacteria culture applied. After 8 weeks of storing it was determined that the content of nitrites and nitrates in the products with the bacteria culture applied was significantly lower than in the products without added strain ($p \leq 0.05$). This phenomenon may be justified with a significantly lower oxidation and reduction potential and higher acidity of the products made with the application of bacterial culture for meat curing (Fig. 2).

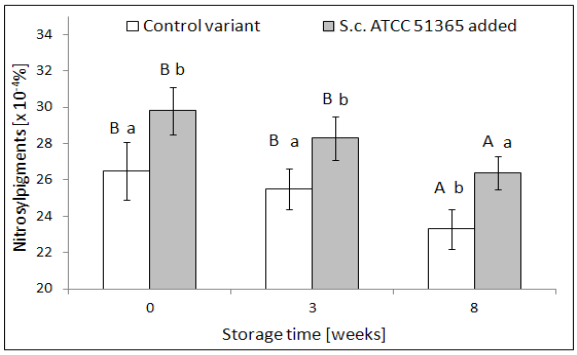
Fig. 2 Changes of redox potential and pH in the model products during storage



Explanatory notes as in Fig 1.

The reduction capacity of the environment affects the speed of nitrite reduction and the nitrosation reactions of hem pigments [10].
In the obtained model products in which *S. carnosus* ATCC 51365 bacteria for meat curing were applied, the significantly higher content of nitrosylpigments was determined as compared to the products without culture ($p \leq 0.05$) (Fig. 3).

Fig. 3 Nitrosylpigments content in the model products during storage



Explanatory notes as in. Fig 1.

The mechanism that could justify the formation of bigger quantity of nitrosylpigments in the products where *S. carnosus* ATCC 51365 was applied may be multidirectional. One of the effects of application of bacteria culture was the lowering of pH value of the finished product. Gøtterup et. al [11] state that there is a strong correlation between the acidification of the environment by bacteria (pH ≤ 5.5), and formation of NO. On the other hand, however, the observed difference in acidity of the model products was minor (0.04 of a unit) and it is hardly probable that this parameter played a decisive role in the quantity of the resulting nitrosylmyoglobin. That is why another mechanism seems to be more probable. One may assume that in the environment of forcemeat, lactic acid produced by *S. carnosus* ATCC 51365 may dissociate rapidly releasing hydrogen ions. The acidic rest reacts with sodium cations that naturally exist in meat, forming sodium lactate. If sodium lactate is added to meat, there is no increase of environment acidity [12, 13], and its role in the creation of pickled meat colour is different than that of lactic acid [13]. It is assumed that sodium lactate stimulates the growth of the level of NADH coenzyme arising from NAD by conversion of lactate to pyruvate by LDH enzyme (lactate dehydrogenase). In effect, the increased quantity of NADH coenzyme reduces metmioglobin to deoxymioglobin more effectively. The increasing quantity of deoxymioglobin contributes to generation of nitric oxide from nitrites in result of the reaction of oxidation-reduction of nitrites with deoxymioglobin. NADH generated with the participation of lactates may also supply nitric oxide in the process of trans-reaction of hem proteins and take part in the reduction of nitrosylmetmyoglobin to nitrosylmyoglobin.
The application of *S. carnosus* ATCC 51365 strain significantly affected the component values of the colour L* a* b* of the model meat product measured after production and after storing (Tab. 1). The statistically significantly higher value of the colour parameter a* and significantly lower colour parameter b* characterising the share of yellow colour and value of colour parameter L* characterising the lightness of the product, were characteristic for the model products made with the application of denitrifying bacteria ($p \leq 0.05$) (Tab. 1).
The performed studies did not show a substantial influence of the applied bacteria strain on the microbiological quality of the model product ($p \leq 0.05$). The total quantity of microorganisms in both experimental variants was on a rather similar level: from 2.36 log CFU/g to 2.79 CFU/g in the whole storing period.

Table 1 Colour parameters L* a* b* of the model products during storage

Sample n=25	Colour parameters		
	L*	a*	b*
After production			
K	$64.25^{b} \pm 0.45$	$7.01^{a} \pm 0.51$	$6.78^{b} \pm 0.32$
S	$63.78^{a} \pm 0.59$	$7.33^{b} \pm 0.61$	$6.18^{a} \pm 0.73$
After 3 weeks of storage			
K	$64.18^{b} \pm 0.51$	$8.18^{a} \pm 0.32$	$5.47^{b} \pm 0.20$
S	$63.03^{a} \pm 0.38$	$8.50^{b} \pm 0.30$	$5.06^{a} \pm 0.25$
After 8 weeks of storage			
K	$64.13^{b} \pm 0.53$	$4.03^{a} \pm 0.24$	$9.32^{b} \pm 0.23$
S	$63.08^{a} \pm 0.52$	$4.58^{b} \pm 0.36$	$8.34^{a} \pm 0.24$

K - the control sample without added *S. carnosus* ATCC 51365 strain; S - sample with added *S. carnosus* ATCC 51365 strain. Mean values by different letters statistically significantly ($p \leq 0.05$)

IV. CONCLUSION

The application of denitrifying bacteria *Staphylococcus carnosus* ATCC 51365 in the process of nitrite meat curing increased the availability of nitrites in meat, by reduction of nitrates resulting from dismutation reaction. It was proved that the application of *S. carnosus* ATCC 51365 for meat curing with nitrites improved the effectiveness of the process and had a positive effect on the colour of the meat product subject to heat processing. The enrichment of the natural meat microflora with the selected denitrifying bacteria strain contributed to the lowering of the level of nitrates in the model product subject to heat processing, as compared to the control variant after production and nitrates and nitrites after the 8-week storing period. The application of *S. carnosus* ATCC 51365 strain in the process of curing of meat destined for making products subject to heat processing is a well-promising direction of modification of technology in order to obtain products of better health quality.

V. REFERENCES

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