Effect of probiotic starter culture on the cell fraction of semi-dried raw sausages

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Abstract

The purpose of this study was to evaluate the effect on structure modification and protein fraction alteration during ripening and drying of semi-dry sausages due to an application of a new lyophilized mixed bacterial starter culture with probiotic activity including the strains *Bifidobacterium longum*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum* and *Micrococcus varians*. Samples were analyzed by SEM and furthermore the changes in amino nitrogen content, hydrolysis rate and the amount of free animo acids were monitored. Designed starter culture application in concentration of 1-1,5 g/kg meat mixture caused greatly *Z*-lines degradation of sarcomeres, myofibrillar proteins solubilization and extraction from *A*- and *I*- regions. The amount of free amino nitrogen and protein hydrolysis increased as higher concentration of elected starter culture was used. In consequence undesirable organoleptic changes were determined and a softer texture was formed that is not typical for this sausage group.

Introduction

In the last decade more and more studies embraced the investigation for new starter cultures with probiotic action application in the raw dried meat products. The use of suitable probiotic strains in the semidried raw sausages production co-operated for the biochemical processes acceleration, improvement of the technological and sensory properties. As result of the passing meat proteins hydrolysis the quantity of the undestroyed muscle fibers abruptly decreased and endomysis and perimysis partial destruction was observed, which contributed to the final products consistency improvement (Koohmaraie M., et al., 2006). Moreover, a possibility for production of new meat products with probiotic action and significant health effect was created (Ammor M., et al., 2007). The probiotic cultures' activity was mainly connected with their influence on the sarcoplazmatic and mono-fibrilic proteins of the filling mass solubility (Fadda, S., et al., 1999). The degree of this hydrolysis was effected by the products drying conditions, also.

The aim of the current elaboration was to establish the influence of the new lyophilized combined starter culture (LCSC) with probiotic action consisting of the strains *Bifidobacterium longum*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum* and *Micrococcus varians* on the protein fraction changes during the drying and ripening of semi-dried sausages.

Material and methods

In the experiment new LCSC of the strains *Bifidobacterium longum*, *Lactobacillus bulgaricus,Lactobacillus plantarum* and *Micrococcus varians* was used, produced by "Lactina" Ltd. – Bankja, Bulgaria in concentration 1, 1.5 and 2.0 g/kg. The experiments were varied out with semi-dried sausage prepared from: 40 % pork breasts, 30 % lean veal, 30 % pork leg, 1.81 % nitrite salt, 0.50 % ground black pepper, 0.10 % sugar, 0.10 % garlic, 0.05 % nut meg, 0.02 % ascorbic acid. The samples were drained at 20 - 24°C and air relative humidity 96 % for 48 h and after that were dried at 22 - 18°C and air relative humidity 90 - 85 %. After the second pressing of the sausages the drying continued at 15°C and air relative humidity up to 85 - 75 %. Three variances of samples were examined, as followed: I variance – only with addition of LCSC; II variance – with addition of LCSC and 0,2 % ascorbic acid; III variance – with addition 0,2 % ascorbic acid and 0,7 % glukono-delta-lacton (GdL).

In parallel with this control samples of semi-dried sausages were produced, for the preparation of which no started culture and additions were used.

To determine the LCSC effect on the protein fraction of the ready sausages state, the changes of the free amino acids and polypeptides quantity, the amine nitrogen and degree of hydrolysis were traced.

For the amine nitrogen determination the method for amine groups' nitrogen estimation by formalin tittring was used, based on the formalin capability to combine the amine groups. The quantity of the free amino acids and polypeptides was found out by the free carboxyl groups' determination. (Антипова, Л. И др., 2004).

Results and discussions

The results from the study on the lyophilized starter culture effect on the polypeptides content in the raw dried sausages was presented in Table 1. It was seen that with the increase of the concentration of starter culture introduced the quantity of the free polypeptides increased in all three variances examined (Table 1).

 Table 1. Polypeptides quantity in semi-dried sausages depending on the concentration of the LCSC and additions introduced (% towards dry content)

Introduced LCSC concentration	I variance	II variance	III variance
(g / kg filling mass)	(without additions)	(with addition of	(with addition of 0,2
		0,2 % ascorbic	% ascorbic acid and
		acid)	0,7 % GdL)
control	3,13 ±0.10	$3,02 \pm 0.08$	3,25±0.12
1 g / kg	$3,63 \pm 0.09$	$3,75 \pm 0.09$	3,81±0.10
1,5 g / kg	3,76±0.10	3,79±0.07	3,88±0.09
2,0 g / kg	3,97±0.12	4,46±0.11	4,35±0.13

It showed that with the increase of the concentration of the LCSC introduced the meat protein hydrolysis in the filling mass for semi-dried sausages increased, which was confirmed by the data for higher degree of hydrolysis (Figure 3).

Similar trend was observed during the investigation of the LCSC effect on the free amino acids content. Their quantity also increased with the increasing of the concentration of the LCSC introduced in all three variances of sausages produced (Figure 1).





Figure 1. Free amino acids quantity (% towards dry content) in semi-dried sausages depending on the concentration of the LCSC introduced.



The results of the experiments for the LCSC effect on the amine nitrogen content and the hydrolysis degree in semi-dried sausages were presented at Figures 2 and 3.

Significant increase of the amine nitrogen quantity was found out in the experimental samples, produced with the addition of LCSC, 0.2% ascorbic acid and 0.7% GdL in comparison with the control ones during the production of which no starter culture and additions were used. (Figure 2).

When starter culture in concentration 1 g/kg filling mass was used, the quantity of the amine nitrogen in variance I was 19,82 mg%, II - 20,72 mg%, III - 19,42 mg %, while when the higher concentration of LCSC of 2,0 g/kg was used, these quantities increased to 23,52 mg %, 24,01 mg % and 23,72 mg %, respectively. It was accompanied by a higher degree of hydrolysis, which increased in variance I from 0,84 % to 1,00 %, in II - from 0,93 % to 1,08 % and in III - from 0,85 % to 1,04 % (Figure 3).

As a result of the electron microscope examinations it was determined that the LCSC used caused strong tearing to pieces the Z-lines of the sarcomeres and myofibrils destruction in the semi-dried sausage. It was ascertained that the LCSC addition in concentration 1g/kg ministers higher degree of hydrolysis of the myofibrils, in consequence of which the solubilization and extraction of myofibrillar proteins from the anisotropic and isotropic sectors was more strongly expressed and embraced bigger spaces (Figure 4).

The analysis of the results from the sensory examinations of semi-dried sausages with higher concentrations of LCSC (1,5 and 2,0 g/kg filling mass) showed deterioration of the appearance, consistency and taste. It was estimated that the samples containing 2,0 g LCSC had softer consistency, side taste and did not possess the characteristic red-brown colour of this type of sausages.





Figure 3. Degree of hydrolysis (%) in semi-dried sausages depending on the concentration of the LCSC and additions introduced.

Figure 4. Electron microscope picture 10000 x of a part of semi-dried sausage with addition of 1 g/kg LCSC without other additions.

When higher quantity of the LCSC was added (2,0 g/kg filling mass) the semi-dried sausages got bitter side taste. As a probable reason was considered the deeper meat proteins dissimilation, which often leaded to bitter polypeptides formation. Usually, the bitter side taste of the meat products was connected with the presence of the hydrophobic amino acids alanine, leucine, isoleucine, valine, proline, phenylalanine and methionine. When the content of these amino acids was higher in the final product there was clearly expressed tendency bitter taste to appear. That is why one possible way to avoid the bitter taste occurrence was to use lower concentrations of LCSC. Then the hydrolytic break-up under the LCSC action stopped at a stage, when the polypeptide chains were still long enough to cover part of the hydrophobic amino acids inside

Conclusions

The results from the experiments for applicability of the new LCSC of strains *Bifidobacterium longum*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum and Micrococcus varians* during the preparation of semi-dried sausages showed that the use of starter culture leaded to free amine nitrogen quantity and the degree of proteins hydrolysis increase in comparison with the control sausage products. It was considered that this was due to the meat proteins proteolysis caused by the influence of the introduced starter culture. The use of LCSC co-operated for the ready sausages structure improvement. The analysis of the results obtained from the complex investigations showed that advisable concentration for introduction of LCSC into semi-dried sausages was 1,0 g/kg filling mass.

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