PROTEOLYTIC EFFECT OF STARTER CULTURES USED IN THE PRODUCTION OF BULGARIAN DRIED-CURED SAUSAGES

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Abstract

Three starter culture combinations including lactic acid bacteria and micrococci strains were studied with the aim of establishing their effect on the protein fraction and amino acid composition of a traditional Bulgarian dried-cured sausage, i.e. Panagyurska Lukanka. The starter cultures included the lactic acid bacteria Lactobacillus plantarum, Lactococcus lactis, Lactobacillus casei and Micrococcus varians supplied by Lactina Ltd. based in the town of Bankya, Bulgaria. The samples were monitored for amino nitrogen content, hydrolysis rate and amount of free amino acids. The analysis results showed that all three starter cultures used had a positive effect on the proteolytic changes and the kind and amount of free amino acids; and the most pronounced changes were observed in the samples made with the starter culture including Micrococcus varians, Lactococcus lactis, and Lactobacillus plantarum.

Index Terms – *dry cured sausage, free amino acids, ripening, starter culture*

I. INTRODUCTION

The proteolytic processes occurring in dried-cured meat products are among the most important biochemical reactions which need to be controlled in order to obtain a quality end product. Proteolysis can be influenced by a number of factors such as breed and age of the animals, muscle kind, presence of cooking salt, factors related to the technology, temperature and curing time applied which have different effects on the muscle protease activity.

Proteolytic activity can be controlled by a suitably chosen starter culture and clearly specified technological parameters which would lead to a naturally preserved dried-cured end meat product of enhanced sensory characteristics and higher nutritive value (Casaburi, A., Di Monaco, S., Cavella, S., Toldra, F., Ercolini, D., Villani, F. (2008).

Various kinds of microorganisms have been used as starter cultures for dried-cured meat products: L.plantarum, L.curvatus, L. carnosus, Micrococus varians, Staphylococcus xylosus, Debaryomices hansenii, etc. (Toldra, F., 2006). During maturation and drying, muscle and microbial proteases demonstrate a complex activity depending on the microorganisms used as starter cultures. Lactic acid microorganisms constitute a group that is widely used in the production of fermented food. Although the proteolytic systems of the lactic acid bacteria applied in the production of dairy products have been well described, information on those used for meat products is still limited. The aim of the present study was to investigate the proteolytic effect of starter cultures including lactic acid bacteria and micrococci used in the production of traditional Bulgarian dried-cured sausages.

II. MATERIALS AND METHODS

With a view to determining the effect of starter cultures on the protein fraction, 3 batches of dried-cured sausages of the Panagyurska Lukanka type were prepared with composition as follows: (per 5 kg of batter): one-grade beef (3.0 kg), lean pork (1.0 kg), pork breast (1.0 kg), salt (0.115 kg), sugar (0.004 kg), nitrate (0.002 kg), black pepper (0.015 kg) and cumin (0.015 kg). The studies were made with three new combinations of starter cultures provided by Lactina Ltd., based in the town of Bankya, Bulgaria:

Variant 1. Micrococcus varians : Lactobacillus casei : Lactobacillus plantarum = 1:1:1;

Variant 2. Micrococcus varians : Lactococcus lactis : Lactobacillus plantarum = 1:1:1;

Variant 3. Micrococcus varians : Lactobacillus plantarum : Lactobacillus casei : Lactococcus lactis = 1:1:1:1.

Control samples were prepared without starter cultures. Test samples 1, 2 and 3 were made by adding the respective starter culture (1,2,3) in a 1 g/kg meat batter proportion ensuring 106 cfu/g. The meat batter was prepared in a cutter machine. It was stuffed in artificial casings with piece length of 20 cm and φ = 45mm. The sausages were placed in a conditioning chamber at a temperature of 22÷24°C and relative humidity of air 90÷95 % for 24÷36 h, then transferred to another chamber where the relative humidity and temperature were gradually reduced from 80÷90 % and 15÷18°C to 80÷75 % and 10÷15°C respectively. Pressing was applied on the 5th and 15th day. Drying continued until the water content reached 30-35 % of the total mass.

The proteolytic effect of the starter cultures used on the protein fraction of Panagyurska Lukanka was determined by a study of the changes in the α – amino nitrogen, the degree of hydrolysis and the free amino acid content. The free α –

amino nitrogen was established using the Wylie and Johnson method. The degree of protein hydrolysis was calculated as the amount of free α – amino nitrogen in relation to the total nitrogen quantity in the sample determined by means of the Kjeldahl method. The AOAA method, 1970, was used for determination of the free amino acid amount and kind.

III. RESULTS AND DISCUSSION

The results of the studies on the effect of bacterial starter cultures on the amino nitrogen content and degree of hydrolysis in dried-cured sausages of the Panagyurska Lukanka type have been presented in fig. 1 and 2.

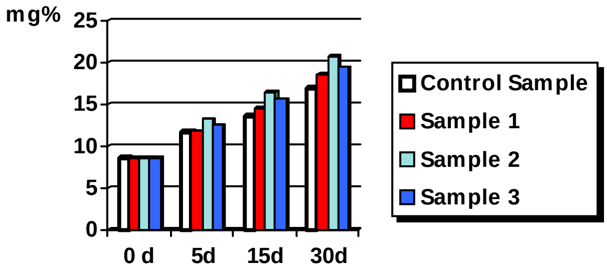

Figure 1. Amino nitrogen content (mg%) in relation to the type of starter culture added

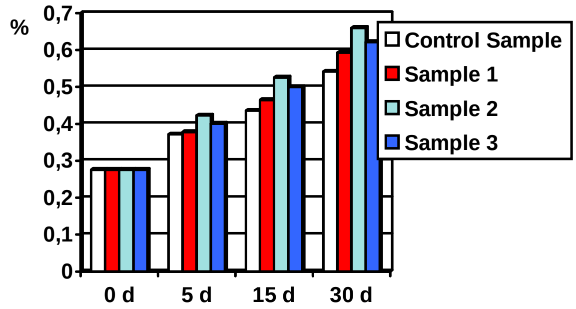

Figure 2. Degree of hydrolysis (%) in relation to the type of starter culture added.

When starter culture Variant 1 was used, the amino nitrogen amount increased to 18,5 mg %, whereas the use of Variants 2 and 3 led to an increase in these quantities up to 20,6 and 19,4 mg % respectively (Fig. 1). This more intensive formation and accumulation of free amino nitrogen in the test samples in comparison to the control samples was probably due to the meat protein proteolysis caused by the effect of the starter culture added. The strains used, especially L.plantarum and L.casei are capable of hydrolyzing sarcoplasmic proteins (Sanz, Y., Fada, S., Vignolo, G., Aristoy, M., Oliver, G., Toldra, F. (1999).

A higher degree of hydrolysis of the protein fraction was also established in the test samples in comparison with the control samples, this degree being most pronounced in the samples made with the Variant 2 starter culture. The results showed that the lactic acid bacteria included in the composition of the investigated starter cultures indirectly contributed to the proteolytic processes in the test sausages. This could have been due to the complex action of muscle and bacterial peptidases and exopeptidases occurring in the last stage of the proteolytic changes during maturation and drying of dried-cured sausages.

The results presented in Table 1 showed that during maturation and drying the total free amino acid amount increased both in the samples made with a starter culture and in the control samples. In the test samples the increase in the amount of free amino acids was significantly greater than that in the control samples. The comparison of the results on the total free amino acid amount in the samples made with starter cultures showed that as early as the 3rd day of the maturation and drying process the free amino acid amount in the samples with starter culture Variant 2 was higher than that in the samples with Variants 1 and 3. A more significant increase in the free amino acid amount was observed on the 15th day of the maturation and drying process, their amount in the test samples being around twice as high as that in the control samples. It was found that in the ready sausages made with starter culture Variant 2, the free amino acid content was around 2.2 times as high as that in the control samples.

We believe that the starter cultures containing lactic acid bacteria and micrococci from Lactina Ltd. contribute largely to the accumulation of free amino acids in dried-cured sausages which results from the combined action of muscle and bacterial peptidases. A number of other studies have also established a higher degree of peptide decomposition to amino acids in meat products caused by microbiological and tissue enzyme activity, however significant differences have been observed in the composition and amount of resultant amino acids (Beriain, M. J., Lizaso, G., and Chasco, J. (2000); Waade, C., and Stahnke, L.H. (1997); Zapelena, M. J., Astiasaran, I., and Bello, J. (1999). According to Molly, K., Demeyer, D. I., Johansson, G., Raemaekers, M., Ghistelinck, M. and Geenen, I., (1997), around 40% of the peptide decomposition to amino acids in meat products is due to the action of the proteolytic enzyme systems of microorganisms.

In all investigated samples, with or without a starter culture, an increase in the amounts of the amino acids leucine, histidine, serine, threonine and asparaginic acid was observed throughout the technological process (Table 1).

A decrease in the arginine and tyrosine amounts was established in the test and control samples, whereas the amounts of the other free amino acids, methionine, isoleucine, and proline, did not change significantly within the period studied.

Table 1 Changes of free amino acid content during the ripening in relation to the starter culture added

Amino acids (mg/100 g dry matter)	Time (days)												
	Filling mass	5 d				15 d				30 d (Final product)			
		Control	Sample 1	Sample 2	Sample 3	Control	Sample 1	Sample 2	Sample 3	Control	Sample 1	Sample 2	Sample 3
Lysine	41.02 ± 1.02	133.70± 3.6	243.62 ± 2.69	397.01 ± 4.09	298.06± 3.61	144.16 ± 4.56	359.02 ± 2.25	402.52 ± 2.97	384.12± 2.55	165.38± 3.17	395.33± 4.50	581.79± 2.80	468.23 ± 3.57
Histidine	230.8 ± 0.42	598.16± 4.39	658.69 ± 6.86	828.24 ± 4.56	766.15 ± 5.21	543.33 ± 3.61	620.77 ± 2.76	720.30 ± 5.63	694.79± 4.52	482.21± 3.73	654.38± 2.96	1441.87± 3.62	896.34 ± 4.26
Arginine	19.4 ± 0.49	63.03± 1.56	ND	ND	ND	46.41 ± 1.55	ND	6.83 ± 0.16	1.21± 0.51	ND	22.12± 1.13	12.34± 0.90	19.25 ± 1.87
Asparaginic acid	9.53 ± 0.28	12.52 ± 0.98	35.47 ± 1.95	39.99 ± 1.97	37.15 ± 0.83	11.26 ± 1.23	98.92 ± 3.68	147.14 ± 3.25	129.16± 4.01	20.84± 2.24	61.80± 2.97	131.79± 2.08	112.37 ± 2.36
Threonine	25.17 ± 0.36	35.36 ± 1.94	81.78 ± 2.87	52.80 ± 1.90	67.29 ± 3.15	53.13 ± 2.90	115.20 ± 3.32	132.47 ± 3.30	121.94± 3.52	72.48± 2.42	148.05± 1.70	129.90± 1.95	132.72 ± 2.06
Serine	30.31 ± 0.62	217.31 ± 3.35	321.78 ± 4.18	209.62 ± 4.84	256. 74 ± 3.84	281.57 ± 3.51	197.03 ± 5.21	412.21 ± 2.73	249.75± 4.21	284.20± 3.96	185.09± 2.26	287.03± 2.80	226.69 ± 2.07
Glutamic acid	64.71 ± 0.45	79.37 ± 0.53	295.87 ± 3.84	222.81 ± 3.71	236.52 ± 0.94	113.42 ± 3.80	404.17 ± 1.34	503.96 ± 4.06	461.78 ± 5.19	198.84± 6.75	552.14± 2.90	447.21± 4.01	495.21 ± 1.13
Proline	94.45 ± 1.12	72.15 ± 3.06	70.97 ± 0.51	83.10 ± 2.24	75.34 ± 1.83	63.65 ± 1.38	103.21 ± 3.92	112.68 ± 4.30	107. 05 ± 2.14	79.78± 3.62	69.60± 1.93	117.71± 4.00	89.54 ± 2.74
Glycine	60.76 ± 1.85	86.28 ± 1.34	74.08 ± 2.60	96.39 ± 2.94	82.31 ± 1.75	73.94 ± 2.47	86.63 ± 0.98	171.12 ± 3.49	134.98 ± 1.25	80.04± 1.66	147.59± 3.97	100.62± 1.21	124.31 ± 2.34
Alanine	180.14 ± 5.45	258.79 ± 4.02	285.94 ± 4.02	335.83 ± 3.53	314.23 ± 2.87	276.24 ± 4.84	313.94 ± 3.33	532.47 ± 3.66	438.36 ± 3.27	305.31± 4.84	253.03± 4.04	275.52± 2.26	255.63 ± 2.81
Cystine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	22.90± 4.29	ND	12.67 ± 1.39
Valine	128.46 ± 2.74	64.44 ± 1.49	129.17 ± 1.45	132.77 ± 4.30	131.02 ±1.67	72.66 ± 3.64	154.32 ± 1.30	136.56 ± 5.93	142.57 ± 3.65	84.44± 2.56	137.74± 3.94	187.66± 2.01	156.87 ± 3.82
Methionine	46.96 ± 2.41	34.16 ± 3.17	59.25 ± 1.77	54.62 ± 2.23	56.14 ± 1.91	33.64 ± 1.67	78.66 ± 1.73	85.24 ± 1.34	71.24 ± 1.29	41.52± 2.42	93.74± 2.53	95.50± 1.55	87.64 ± 3.95
Isoleucine	79.32 ± 1.99	47.74 ± 2.09	79.61 ± 2.01	71.42 ± 2.45	74.92 ± 2.14	50.00 ± 3.35	113.70 ± 1.60	107.84 ± 2.50	110.72 ± 1.87	59.67± 3.67	144.03± 1.39	135.70± 5.34	136.97 ± 1.42
Leucine	167.42 ± 3.78	102.35 ± 4.21	208.50 ± 2.94	200.89 ± 5.68	204.36±3. 88	104.73 ± 2.93	265.53 ± 2.66	283.57 ± 3.41	271.36 ± 2.57	127.60± 2.09	329.20± 3.02	336.26± 1.24	330.97 ± 3.12
Tyrosine	118.84 ± 0.40	55.16 ± 3.12	31.79 ± 1.28	75.54 ± 7.50	52.69 ±5.30	42.10 ± 2.99	38.22 ± 0.98	23.79 ± 1.46	31.56 ± 4.14	40.65± 2.16	88.30± 2.79	78.88± 1.72	81.67 ± 2.69
Phenylalanine	93.91 ± 0.58	56.81 ± 0.68	71.53 ± 1.30	105.67 ± 2.88	94.11± 1.33	51.19 ± 2.38	133.30 ± 4.37	147.99 ± 2.04	139.84 ± 3.47	73.71± 1.36	154.23± 2.19	160.33± 4.33	158.32 ± 3.62
Total	1391.70 ± 1.60	1919.33 ± 5.34	2648.05 ± 6.17	2906.80 ± 3.15	2747.03 ± 2.68	1961.25 ± 8.89	3091.62 ± 1.22	3926.69 ± 3.92	3490.43 ± 3.01	2116.67± 8.03	3457.27± 1.48	4520.11± 1.28	3785.40 ± 2.45

In the ready products of both the test and the control samples the histidine amino acid content was the highest. In the sausages made with the Variant 2 starter culture, the amount of this amino acid was around 2,2 times as high as the amount in the samples made with the Variant 1 starter culture and about 3 times as high in comparison with the control samples made without a starter culture.

High amounts of lysine (581.79 mg/100g), glutamine acid (447.21 mg/100g), and leucine (336.26mg/100g) were found in the ready products made with the addition of starter culture Variant 2. The same amino acids but in amounts lower than the ones mentioned were also established in the ready products made with the addition of starter culture Variants 1 and 3. In the control samples without a starter culture, the amounts of alanine, serine and glutamine acid were higher. The arginine and cystine amounts were the lowest both in the samples with starter cultures and in the control samples.

IV. CONCLUSION

The analysis of the results obtained showed that the three starter cultures from Lactobacillus plantarum, Lactobacillus casei, Lactococcus lactis and Micrococcus varians strains had a significant effect on the amino acid profile of the investigated samples of dried-cured sausages. The higher amount of free amino acids formed in the samples with the Variant 2 and 3 starter cultures was probably due to the proteolytic and aminopeptidase activity demonstrated by the micrococci and lactococci strains in the lyophilized starter culture. The increase in the free amino acid amount after addition of micrococci containing starter culture was reported by De Masi, T. W., Wardlaw, F. B., Dick, R. L. and Acton, J. C. (1990) and confirmed by the results of our research.

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