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Original scientific paper

ANTIMICROBIOLOGICAL EFFECT OF EXTRACTS FROM SPICES DURING PRODUCTION OF SEMIDURABLE NATIONAL SAUSAGES

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In this paper are given the test results of influence of various concentrations of extracts of spices on microbiological analysis for semidurable national sausage. Examined is the impact of extract of garlic and basil at concentrations of 0.1, 0.2 and 0.3 g of extract per kg mixture. For this purpose four samples are prepared: one is a control sample without adding an extract, and the others are by adding 0.1, 0.2 and 0.3 g extract of basil and extract of garlic per kg mixture. The presence of *Proteus, Clostridia, E. coli, Salmonella*, *Listeria monocytogenes* and total number of bacteria was examined. From the beginning to the end of the examination none of the mentioned bacteria were found. Total number of bacteria was established, which are mostly bacilli. In control samples compared with the other samples during storage of the sausages, total number of bacteria was greater. The smallest total number of bacteria from all tested samples was found in the sample with the addition of 0.3 g garlic extract per kg mixture compared with basil extract which indicates a strong antibacterial effect of garlic extract. Sensory characteristics, like taste and smell are better in the sample with the addition of 0.3 g extract of basil and extract of garlic per kg mixture compared with other tested samples. Although, the samples with the addition on basil extract had better results, concentrations on garlic extract which we used, have no negative effect on taste and smell.

Key words: sensory characteristics; microbiological analysis; garlic extract; basil extract: semidurable sausage

АНТИМИКРОБИОЛОШКИ ЕФЕКТ НА ЕКСТРАКТИ ОД ЗАЧИНИ ВО ПРОИЗВОДСТВОТО НА ПОЛУТРАЕН НАРОДЕН КОЛБАС

Во трудот се дадени резултатите од испитувањата на влијанието на различни концентрации на екстракти од зачини врз микробиолошката слика на полутраен грубо иситнет барен народен колбас. Испитувано е влијанието на екстракт од босилок и лук во концентрации од 0,1, 0,2 и 0,3 g екстракт на kg смеса. За таа цел се приготвени по четири проби од кои едната е контролна, без додаток на екстракт, а другите се со додаток на 0,1, 0,2 и 0,3 g екстракт од босилок и екстракт од лук на kg смеса.. Беше испитувано присуството на: *Proteus*, *E. coli, Clostridia, Salmonella, Listeria monocitogenes* и вкупен број бактерии. Кај сите групи испитувани колбаси со додаток на екстрактот од босилок и екстрактот од лук од почетокот до крајот на испитувањето не се утврдени *Proteus, Escherichia, Clostridia, Salmonella* и *Listeria monocitogenes*. Утврден е вкупен број бактерии кои се претежно бацили. Кај контролната проба во споредба со другите проби вкупниот број бактерии за време на чувањето на колбасите беше поголем. Најмал вкупен број бактерии од сите испитувани проби беше утврден кај пробата со додаток на 0.3 g. екстракт од лук на kg смеса во споредба со екстрактот од босилок, што укажува на посилното антибактериско дејство на екстрактот од лук. Во однос на сензорните особини вкус и мирис подобри сензорни оценки добија пробите со додаток на 0,3 g екстракт од босилок и екстракт од лук на kg смеса во споредба со другите испитувани проби .Иако нешто подобри резултати во однос на сензорните особини вкус и мирис имаше кај пробите со додаток на екстракт од босилок, концентрациите на екстрактот од лук кои ние ги користевме немаа негативно влијание на сензорните особини вкус и мирис.

Клучни зборови: сензорни особини; микробиолошка анализа; екстракт од лук; екстракт од босилок; полутраен колбас

INTRODUCTION

Because of the specific composition, meat and meat products belong to the group of high risk, very perishable products (Devatkal et al., 2012). Quality, and therefore durability of the products can worsen during their storage – storage due to the growth of microorganisms and the oxidation of lipids. In the meat industry, retail objects and consumers, rotten meat and meat products is economic loss which can amount to over 40% (Sperber, 2010).

One of the reasons for rotten meat and meat products is their contamination with microorganisms. The growth of microorganisms in meat and meat products can be controlled or minimized with impeccable hygiene during production process, using vacuum packaging, properly performed technological process for the production of meat products and using natural or synthetic additives and supplements their production (Mielnik et al., 2003). For a long time, natural spices and their extracts are subject to study, because they are natural resources which have antimicrobial activity and are easily accepted by consumers (Kuzelov et al,, 2014; Dragoev, 2004; Nguefack et al., 2004; Nebedum et al., 2009; Sanchezet et al., 2010; Popović et al., 2007; Venu et al., 2012; Yin M. C. and Cheng W. S., 2003; Burt, 2004; Ashok Kumaret et al., 2011; Ankri, S. and D. Mirelman, 1999; Kumar and Berwal, 1998; Prasad G., Saharma V. D., 1981; Sivam G. P., 2001; O'Gara et al., 2000; Vlajić et al., 2012; Vlajić et al., 2013; Bekemblia, 2004; Yin et al., 2003).

According to the data from the literature and practical knowledge, extracts of basil and garlic are very little included in the meat industry. Mostly they are used as components in mixture of spices. Because, there is very little literature on the impact of data extracts basil and garlic on the quality and durability of semidurable sausage, the aim of our study was to examine the effect of different concentrations of the extract of basil and extract of garlic on the microbiological analysis and sensory characteristics on semidurable national sausage.

MATERIAL AND METHOD

As material for examination we have used the semidurable sausage of the type produced home – national sausage. Sausage was produced in compliance with all veterinary sanitary regulations which apply in the Republic of Macedonia. For the production of the sausage the following raw material was used: beef second category (25%), pork second category (20%), fat tissue (30%), meat steak (10%) and hard water-ice (15%): per 1 kg is added 18 grams of nitrite curing salt and 3 grams phosphate product, emulsifier 0.020 g/kg, spices mixture semidurable national sausage 0.040 g/kg (*Koleks, Ljubljana, Slovenia*). The mixture is stuffed into small pork's intestine. For the experiment four samples were prepared. At first, samples were prepared with adding extract of basil:

– Sample 1 – control sample without adding extract of basil.
– Sample 2 – sample by adding 0.1 g extract of basil per kg mixture.
– Sample 3 – sample by adding 0.2 g extract of basil per kg mixture.
– Sample 4 – sample by adding 0.3 g extract of basil per kg mixture.

After, prepared samples with adding extract of basil and full examination of samples, were prepared samples with adding extract of garlic:

– Sample 1 – control sample without adding extract of garlic.
– Sample 2 – sample by adding 0.1 g extract of garlic per kg mixture.
– Sample 3 – sample by adding 0.2 g extract of garlic per kg mixture.
– Sample 4 – sample by adding 0.3 g extract of garlic per kg mixture.

The extracts of basil and garlic are produced in the company of ECOL Canada. Extracts were 100% pure, their microbiological picture was spotlessly and they were applied in production grinding the mixture. After filling and leaving the water to come out of the sausage, it was thermically prepared. Thermal processing is implemented by the following formula: 35 minutes drying, 20 minutes smoked at 62ºC, 35 minutes of boiling at 78ºC or until the center of the product does not reach a temperature of 69 to 72°C. After thermal processing, it was vacuumed with vacuum machine Vebomak. After vacuum packaging, sausage was stored in a chamber at a temperature of +4^{o}C. During storage on the 1st, 10th, 20th, 30th, 40th and 50th day of production, sausages were examined in view of microbiological analysis. Sensory analyses of the sausages were conducted of the first day and 50th day of production.

Microbiological analysis

For bacteriological examination, material was taken from each sample – 20 g of material, which before planting is homogenized with 180 ml steriled is tilled water from which is made other dilu-

tions. The number of bacteria is in log/CFU/g. Microbiological tests were performed as examining the presence of *Proteus, Clostridia, E. coli, Salmonella*, *Listeria monocytogenes* and total number of bacteria. *Proteus* (brilliant green 37°C/ 24–48 h), *E. coli* (brilliant green bile lactose broth 42°C/24–48 h), *Clostridia* (blood agar 37°C/48 h), *Salmonella* (bismuth sulphite agar 37°C/24–48 h), *Listeria monocitogenes* (Fraser broth base Palcam agar, Oxoid) ISO 11290/2010, total number of bacteria (nutrient agar 37°C/24–48h) ISO 4833/ 2008.

Sensory examination

The evaluation was performed by a 5 experienced specialists following sensory attributes: external appearance, appearance of cut, color section, smell, taste, consistency and appearance of drain in vacuums samples. Evaluators were served bread and water for rinsing-cleaning the mouth after every test samples. Sensory analysis was obtained with using 9 step scale for testing of sensory properties of meat and meat products established by VNIMP Moscow.

Statistical processing

The obtained results have been processed mathematically statistically by determining the medium value, measures of variation, analysis of variance, coefficient of variation and statistical significance (EXCEL MS Office 2003).

RESULTS AND DISCUSSION

The results of the microbiological analysis of all samples of sausages are given in Tables 1 and 2. The Tables show in all simples of tested sausages from the beginning to the end of the test, *Proteus*, *Clostridia, E. coli, Salmonella*, *Listeria monocytogenes* were not determined. The total number of bacteria was determined which are mostly bacillus. Total number of bacteria is greater during storage of sausages in the control group compared with other samples. There are no statistically significant differences between the samples examined in terms of microbiological analysis of the tested sausages.

Popović and Nikšić (2007) and Nebedum et al. (2009) say that basil extract leads to a reduction of the initial number of bacteria especially with *Listeria monocytogenes.* This effect can be explained by the action of essential oils and aromatic compounds that are found in the extract. Nguefack et al. (2004) investigated the antibacterial activity of extracts obtained from various plants (*Cymbopogon citratus, Ocimum basilicum, Ocimum gratissimum, Thymus vulgaris* and *Zingiber officinale*), i.e. their antibacterial activity against *Staphylococcus aureus, Listeria monocytogenes, L. innocua* and found tha tall tested extracts showed antibacterial activity in different degree. The weakest antibacterial activity showed extracts of *Zingiber officinale* and *Ocimunum basilicum* and the strongest of *O. gratissimum, C. citratus*. Sanchez et al. (2010) investigated the antimicrobial activity of extracts basil (*Ocimum basilicum*), cactus (*Opuntia ficus*) and sweet acacia (*Farnesiana* L.) and found these extracts are most active against *V. cholera*.

Table 1

Microbiological analysis for four tested samples sausages by adding extract of basil during storage in vacuum (Log /CFU g.)

Days	Control	Sample 1	Sample 2	Sample 3
1	1.69	1.68	1.66	1.78
10	1.77	1.63	1.73	1.11
20	1.84	1.73	1.75	1.14
30	1.88	1.73	1.77	1.20
40	1.92	1.77	1.80	1.20
50	1.95	1.79	1.89	1.23

Table 2

Microbiological analysis for four tested samples sausages by adding extract of garlic during storage in vacuum (Log /CFU g.)

Days	Control	Sample 1	Sample 2	Sample 3
1	2.14	2.07	2.0	1.70
10	2.14	2.11	2.07	1.02
20	2.14	2.11	2.11	1.08
30	2.17	2.14	2.14	1.10
40	2.19	2.17	2.14	1.15
50	2.17	2.17	2.14	1.10

The extracts of these plants disrupt the cell membrane of *V. cholera,* causing increased thinness of the membrane, reduction of cytoplasmic pH value and hyperpolarization of the cell membrane. Kh. I. Sallam et al. (2009) examined antibacterial effect of fresh garlic, garlic in powder and

extract garlic in chicken sausages stored at a temperature of 3°C. They found that fresh garlic, garlic in powder and garlic extracts have antibacterial effect and continued period of keeping of sausages to 21 days. Vlajić et al. (2012) examined antibacterial effect of extract garlic and found that garlic extract has strong antibacterial effect.

Bekemblija et al. (2004) examined the impact of extracts from garlic and onions on *Staphylococcus aureus*, *Salmomella enteritidis* and fungi *Aspergilus niger, Penicillium cuclopium, Fusarium oxysporum*, they established that garlic extract was acting with bacteria and the fungi (antibacterial and antifungal), and extract onions antibacterial was acting only to *Salmomella enteritidis*. Yin M. C. and Cheng W. S. (2003) says that extract of garlic reduces the evolution *of Bacillus subtitles, Bacillus cereus, Clostridium botulinium* type A, *E. coli, Lactobacillus plantarum, Staphylococcus aureus* and *Salmonella*, and from the fungi evaporative compounds of garlic reduces the evolution of *Candida albicans* evolution and *Pencillium.* Based on the above said, the results we obtained in terms of microbiological condition are probably due to the relatively small antibacterial effect of basil extract and larger antibacterial activity extract of garlic, the effects of high temperature during thermal processing and vacuuming of sausages.

Based on the presented results (Tables 3 and 4) it can be seen that immediately after the end of production and storage, in all groups tested sausages with the extract of basil and extract of garlic had acceptable sensory properties. In terms of the parameter occurrence of liquid, immediately after manufacture, in all groups tested sausages with the extract of basil did not have an appearance of meat drain. At the end of the production in control sample, there was a small amount of liquid in three samples, in sample 1 three samples, in sample 2 two samples, in sample 3 one sample. This is probably a result of the beginning of putrescence of the tested sausages (appearance of slicing). In tested sausages with extract of garlic immediately after manufacture and at the end of storage did not have an appearance of meat drain in any sample.

Table 3

Results of sensory evaluation immediately after the end of production and at the end of storing sausages with extract of basil (X ± Sd)

Sensory properties	After the end of production				At the end of storing sausages			
	C	I	II	III	C	I	II	III
External appearance	7.4±0.25	7.42±0.28	7.7±0,22	7.78±0,25	6.6±0.28	.6.78±0.22	7.4±0.02	7.45±0;.12
Appearance of crosssection	7.4±0.40	7.22±0.52	7.6±0.45	7.80±0.25	5.8±0.25	6.45±0.42	6.6±0.18	7.12±0.28
Color of crosssection	6.6±0.40	6.52±0.42	6.2±0.20	7.12±.0.45	6.8±0.45	6.88±0.45	7.0±0.20	7.25±0.22
Smell	6.,4±0,28	7.48±0.10	8..2±0.25	9.52±0.12	6.6±0.28	7.25±0.28	8.5,±0.22	8.95±0.10
Taste	6,6±0.22	6,78,±0.12	8.8±0.28	9.22±0.10	6.4±0.50	6.42±0.25	8.5,±0.22	8.7±0.20
Consistency	7.2±0.28	7.22±0.28	7,2±0.42	7,45±0.20	6.6±0.25	7.28 ±0.20	7.0±0.20	7.22±0.20
Appearance of meat drain	/	/	/	/	In 3 samples	In 2 samples	In 2 samples	In 1 sample

Table 4

Results of sensory evaluation immediately after the end of production and at the end of storing sausages with extract of garlic (X±Sd)

Sensory properties	After the end of production				At the end of storing sausages			
	C	I	II	III	C	I	II	III
External appearance	7.48±0.20	7.45±0.18	7.75±0.28	7.80±0.12	6.6±0.20	6.85±0.25	7.48±0,08	7.55±0.18
Appearance of crosssection	7.45±0.12	7.28±0.40	7.72±0.40	7.85±0.28	5.9±0.22	6.58±0.25	6.72±0.12	7.28±0,20
Color of crosssection	6.80±0,12	6.72±0,15	6.50±0.15	7.20±0.28	6,8±0,45	6.92±0.15	7.20±0.12	7.48±0.18
Smell	6.55±0.12	7,40±0.12	8.45±0.15	9,45±0.18	6.42±0.15	7,25±0,28	8.15,±0.18	8,90±0.12
Taste	6.45±0.15	6.58±0.22	8.72±0.20	9.10±..22	6.28±0.18	6.28±0.42	8.42,±0.18	8.52±012
Consistency	7.45±0.28	7.28±0.20	7.40±0.15	7.55±0.22	6.8±0.12	7.42 ±0.15	7.22±0.12	7.50±0.28
Appearance of meat drain	/	/	/	/	/	/	/	/

In all sensory properties there are no statistically significant differences between tests examined (treated with extract of basil and extract of garlic) except in sensory attributes – taste and smell. Smell in sample 3 treated with extract of basil immediately after production and eventually won by keeping the major grades from the smell in other examined samples. The differences between the sample 3 (9.52 ± 0.12 and 8.95 ± 0.10) and the control (6.40 ± 0.28 and 6.60 ± 0.28) and sample 2 (8.2 ± 0.25 and 8.5 ± 0.22) and the control (6.40 ± 0.28 and 6.60 ± 0.28) immediately after production and at the end of storage was statistically significant ($p < 0.001$).

Sensory characteristic – taste in sample 3 treated with extract of basil immediately after production and eventually won by keeping the major grades (9.22 ± 0.10; 8.7 ± 0.20) from the taste in other examined samples and the difference is statistically significant ($p < 0.001$). Sample 2 immediately after production and eventually won by keeping the major grades (8.8 ± 0.28 and 8.5 ± 0,22) in terms of control sample (6,6 ± 0.22 and 6.4 ± 0.50) and sample 1 (6.78 ± 0.12 and 6.42 ± 0.25) and the difference is statistically significant ($p < 0.001$). These higher grades in sensory properties like taste and smell in sample 2 and simple 3 are as a result of the contribution of basil extract in semidurable sausage of the type produced home – national sausage.

Smell in sample 3 treated with extract of garlic immediately after production and eventually won by keeping the major grades from the smell in other examined samples. The differences between the sample 3 (9.45 ± 0.18 and 8.90 ± 0.12) and the control (6.55 ± 0.12 and 6.42 ± 0.15) and sample 2 (8.45 ± 0.15 and 8.15 ± 0.) and the control (6.55 ± 0.12 and 6.42 ± 0.15) were statistically significant ($p < 0.001$). Sensory characteristic taste in sample 3 treated with extract of garlic immediately after production and eventually won by keeping the major grades (9.10 ± 0.22 and 8.52 ± 0.12) from the taste in other examined samples. Sample 2 immediately after production and eventually won by keeping the major grades (8.72 ± 0.20 and 8.42 ± 0.18) in terms of control sample (6.45 ± 0.15 and 6.28 ± 0.18) and sample 1 (6.58 ± 0.22 and 6.28 ± 0.42) and the difference is statistically significant ($p < 0.001$).

Sensory characteristics, taste and smell, in samples with extract of garlic got something better grades of the samples with basil extract, but concentrations of garlic extract which we used in tested samples had no negative impact on the sensory characteristics although garlic extract has a strong aroma.

CONCLUSIONS

According to the above written we can conclude the following: Garlic extract has stronger antibacterial effect compared to extract basil. The best results are received by sample by adding 0.3 g/kg extract of garlic. Taste and smell in samples with extract of garlic got something better grades of the samples with basil extract. Concentrations of garlic extract have a strong aroma, which we used in tested samples had no negative impact on the sensory characteristics, taste and smell, and at low concentrations is desirable to apply in the production of sausages as a substitute for natural garlic.

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