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HYGIENA ALIMENTORUM XXXII

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and

Under the auspices of Ministry of Agriculture and Rural Development of the Slovak Republic and within the World Veterinary Year

"MEAT AND MEAT PRODUCTS – PRODUCTION, SAFETY AND QUALITY"

May 11–13, 2011

Štrbské Pleso – Hotel Patria

The Slovak Republic

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AUTHENTICATION OF FOOD OF ANIMAL ORIGIN USING REAL-TIME PCR METHOD

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ABSTRACT

The molecular techniques developed in the last two decades enabled the development of authentic and reliable methods for identification of meat species. These techniques are promising and may overcome many disadvantages of conventional methods. Polymerase chain reaction (PCR) is the most commonly used technique in many areas of molecular biology because of its sensitivity, specificity and the ability to detect even one copy of DNA sequences from a single cell sample. Many analytical methods are used for species identification of foods of animal origin as well as for their authentication. In our study we applied the method of real-time PCR using TaqMan® probes. We focused on predesigned molecular-genetic marker of the common carp (*Cyprinus carpio*) which comes from the mtDNA control D-loop area. We analyzed its presence in DNA isolates from 15 kinds of freshwater fish, diluted to 10 % concentration, using a TaqMan real-time PCR method.

Key words: *Cyprinus carpio*; PCR; TaqMan® probe; TaqMan real-time

INTRODUCTION

Meat products sold for public consumption must be clearly marked to make it clear what kind of meat they contain. However, inadvertent errors or fraudulent labelling can be detected leads to poor quality product. One of the most occurring forms of counterfeiting for economic gain is the addition of minced pork. Counterfeiting pork is undesirable for vegetarians for religious reasons as well as due to the possible introduction of allergens, bacteria and parasites (2).

In addition to possible economic loss, correct species identification is important for consumers and for other reasons, such as health of persons who may have specific food allergies, or religious and dietary restrictions. To identify these scams and protect the consumer it is necessary to develop reliable and simple tools to facilitate routine control throughout the food chain (5).

Methods for identification of meat are also based on DNA analysis. Compared with protein-based techniques, DNA techniques have proven to be reliable because DNA is stable under conditions of high temperature, pressure and chemical treatment employed during preparation of certain foods (1, 3).

MATERIALS AND METHODS

We isolated DNA from 15 species of freshwater fish (Table 1) according to the protocol NucleoSpin® Food Isolation Kit (Macherey-Nagel) for isolation of genomic DNA from food. DNA samples were then diluted to 10 % concentration and analyzed by capillary cycler LightCycler® 1.5 (Roche) using LightCycler software version 4.5 software (ROCHE).
The designed primers and TaqMan probe are specific for all lines of a gene common carp (*Cyprinus carpio*). Primer pairs were prepared in final form and TaqMan probe designed by (ROCHE, Slovakia). Sequences of primers and TaqMan probes were as follows:
D – Loop F (5´-CATCTGGTTCCTATTTCAGGGA-3´),
D – Loop R (5´-GGCACTATGTAAGGATAAGTTGAACT-3´),
TM^{LNA} (LC640-TGCACTTGAGATAAAAGTATGTAA+T+T+CT-BBQ).

We used a kit LightCycler® TaqMan® Master (ROCHE). PCR cycle, starting with pre-incubation at 95 °C for 10 minutes. It was

Table 1. Selected fish species

Sample No.	In Slovak	In Latin
1	Pstruh potočný	*Salmo trutta fario*
2	Hlaváč pásoplutvý	*Cottus poecilopus*
3	Lipeň tymiánový	*Thymallus thymallus*
4	Pstruh dúhový	*Oncorhynchus mykiss*
5	Sumček čierny	*Ameiurus melas*
6	Úhor sťahovavý	*Anguilla anguilla*
7	Šťuka severná	*Esox lucius*
8	Sumec veľký	*Silurus glanis*
9	Jeseter malý	*Acipenser ruthenus*
10	Ostriež riečny	*Perca fluviatilis*
11	Pleskáč vysoký	*Abramis brama*
12	Jalec hlavatý	*Leuciscus cephalus*
13	Mrena severná	*Barbus barbus*
14	Podustva severná	*Chondrostoma nasus*
15	Pstruh dúhový	*Oncorhynchus mykiss*
16	Kapor obyčajný (pos. control)	*Cyprinus carpio*
17	CYBR H_2O (neg. control)	

subsequently repeated 45 cycles with a temperature profile: 10 sec at 95 °C, 30 sec at 63 °C, 2 sec at 72 °C, followed by measurement of fluorescence. The final cooling was for 30 sec at 40 °C.

RESULTS AND DISCUSSION

The Fig. 1 shows the earliest possible increase in fluorescence intensity and thus exceeded level of nonspecific background in the positive control (Sample 16 – *Cyprinus carpio*) in the 17th cycle of the PCR reaction. The fluorescence intensity corresponds to the shape of the curve, which passes from exponential phase to the linear growth phase. For Samples 3 (*Thymallus thymallus*), 4 (*Oncorhynchus mykiss*), 6 (*Anguilla anguilla*), 7 (*Esox lucius*) and 11 (*Abramis brama*) the nonspecific background levels were exceeded to about 40th cycle of PCR reaction, which is already the late phase of PCR amplification in which we can clearly demonstrate the specificity and affinity of the studied species with the positive control (*Cyprinus carpio*). In the Samples 1 (*Salmo trutta fario*), 2 (*Cottus poecilopus*) 5 (*Ameiurus melas)*, 8 (*Silurus glanis*), 9 (*Acipenser ruthenus*), 10 (*Perca fluviatilis*), 12 (*Leuciscus cephalus*), 13 (*Barbus barbus*), 14 (*Chondrostoma nasus*), 15 (*Oncorhynchus mykiss*), and 17 (CYBR H_2O – negative control) there have been no changes in fluorescence intensity, so these samples did not create the characteristic curve whether in their exponential or linear phase, i. e. there were excess levels of nonspecific background (Table 1).

PCR methods, including commercial kits are suitable to detect the authenticity of fish and fish products, which demonstrated in his work Gil (4) and, if it can be used to protect customers against counterfeit products.

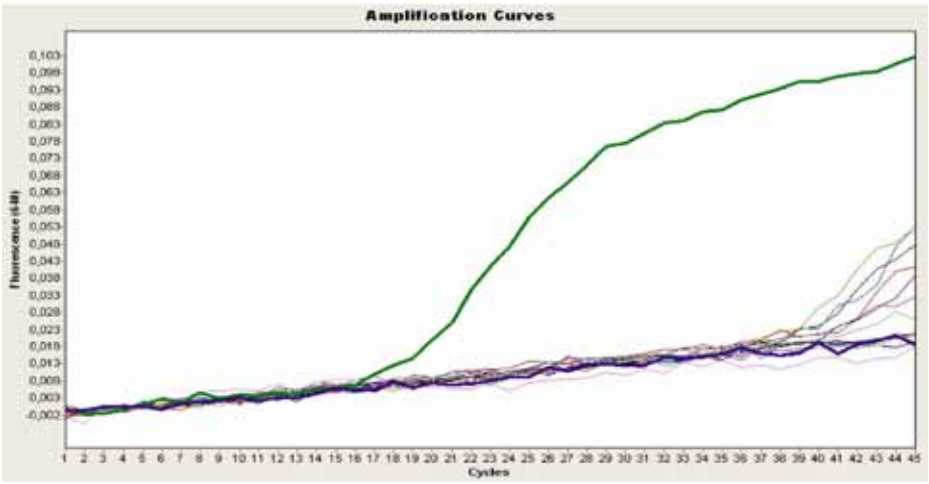

Fig. 1. The curves of the fluorescence during the PCR cycle in individual samples

CONCLUSION

The TaqMan real-time PCR method, using TaqMan probe, is a very sensitive and reliable way of authentication of food of animal origin. Our results confirmed suitability of this method for species identification of the common carp (*Cyprinus carpio*). Already in the 17th cycle of PCR amplification we were able to detect the presence of gene *Cyprinus carpio* in the positive control. For the Samples 3, 4, 6, 7, 11 we also observed a specific curve due to the increase in fluorescence. However, this increase started only after the 40th cycle, when there could be some cross-reactions and the resulting curve might not be specific for the gene studied in the common carp. For this reason we suggest modification of the method in terms of reducing the number of PCR cycles in the capillary cycler to 40.

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COLOUR PARAMETERS OF DFD BEEF MEAT

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ABSTRACT

The aim of our study was to evaluate the colour of DFD and normal beef meat by CIELAB system and to analyse haeme pigments in *Musculus gluteus medius*. Colour parameters (L*, a*, b*) and haeme pigments were measured in DFD beef (pH over 6.2) and normal beef meat (pH < 5.8). Colour parameters and values of pH were measured during 48 hours. We found significant differences (P < 0.001) between DFD and normal beef meat in all pH values and all colour parameters during 48 hours. DFD beef meat had the lowest L* value, a* value and b* value, but the highest pH value.

Key words: beef meat; DFD; L *; a *; b *

INTRODUCTION

The greatest incidence of DFD meat is found in slaughtered bulls fattened in tethering. If animals are housed individually and transported freely together with other animals, there is a lot of fighting for position in the group which leads to excessive fatigue (1, 3). The bulls exhibit physical exhaustion and sexual aggression. In cows, heifers and steers the frequency of DFD quality variations is significantly lower due to their calmer temperament (7).

The DVD quality deviation is assessed sensorially, the meat is very dark, sticky and dry in the sense of absence of fresh cut meat juice due to superior binding capacity of DFD meat. The pH_{ult} value is a reliable criterion for an objective assessment which is in terms of our technology practically identical with pH_{24}. With DFD meat the pH_{24} values arehigher than 6.20. This criterion reliably indicates the worst quality DFD meat, which is associated with reduced shelf life, easy perishability and contamination with microorganisms (4, 7).

The colour is very obvious characteristic for the consumer in assessing the quality of meat and meat products as it is connected with many properties. It is mainly determined by the content of haeme pigments, especially myoglobin content (5, 6, 8).

The aim of this study was to evaluate the colour of DFD beef in comparison with normal beef focusing on the content of haeme pigments affecting the overall colour of meat.

MATERIALS AND METHODS

Samples for the experiment came from the beef – leg parts (*Musculus gluteus medius*). Based on the pH and colour were selected 9 pieces that suited the condition of DFD meat defects. Samples of normal meat were selected for comparison from 9 pieces, which were similar in age (21–29 months), weight (average weight 360 kg) and especially sex (bulls). Samples (approx. 300 g) were collected from each piece of muscle tissue. The pH and colour were measured 24 hours after the slaughter (ultimate value) – marking pH_{0h} equals pH_{ult}, also the colour parameters. Subsequent measurements were performed after 24 and 48 hours under considerable variations in the "maturation".

Muscle pH was measured by inject electrode (Double Pore, Hamilton, USA) using a digital pH-meter WTW 340i (WTW GmbH, Germany). Colour was measured by a spectrophotometer Konica Minolta CM 2600d (Minolta, Japan). Each sample was measured 5 times. The samples were evaluated by a CIELAB system. The content of haeme pigments was determined by Hornsey method (2) measuring the absorbance at 640 nm. The results were

Table 1. Values of pH, L*, a*, b* within 48 hours and content of haeme pigments for DFD and normal beef

Beef meat		DFD		Normal		P value
Parameters		Mean	± SD	Mean	± SD	P <
pH	0h	6.397	± 0.29	5.451	± 0.07	0.001
	24h	6.443	± 0.29	5.477	± 0.07	0.001
	48h	6.462	± 0.31	5.507	± 0.06	0.001
L*	0h	30.249	± 1.59	36.745	± 3.99	0.001
	24h	30.279	± 2.34	38.945	± 2.56	0.001
	48h	29.715	± 1.85	39.215	± 2.98	0.001
a*	0h	7.627	± 1.47	12.105	± 2.26	0.001
	24h	8.209	± 1.21	13.412	± 1.51	0.001
	48h	8.349	± 1.67	12.608	± 1.75	0.001
b*	0h	5.751	± 1.24	10.999	± 2.16	0.001
	24h	6.957	± 1.62	12.907	± 1.00	0.001
	48h	6.866	± 1.78	12.828	± 1.12	0.001
Content of haeme pigments [mg.g^{-1}]		2.070	± 0.40	2.503	± 0.68	N.S.

NS – not significant (P ≥ 0.05); SD – standard deviation

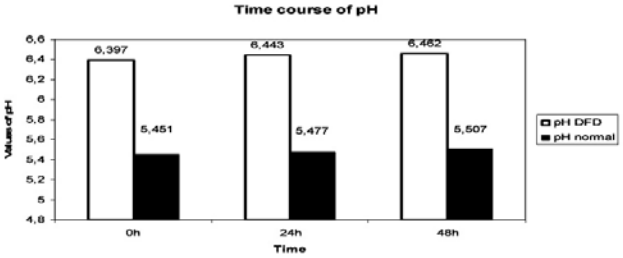

Fig. 1. The course of pH measurement within 48 hours

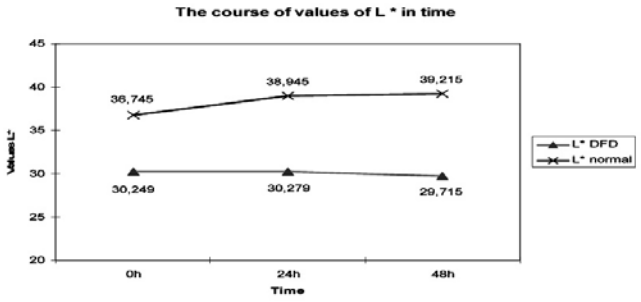

Fig. 2. Course of L* values within 48 hours of measurement

processed by statistical software STATISTICA CZ 7 (StatSoft, Prague, CR).

RESULTS AND DISCUSSION

Table 1 shows the measured colour values (L*, a*, b*) and pH within 48 hours, both in normal and DFD beef meat. Differences between all these parameters were significant (P < 0.001). Contents of haeme pigments in both types of meat were similar, i.e. the haeme pigments did not affect the colour of DFD and normal beef. Our results showed that the colour of DFD meat was darker, but less red and less yellow, as all values of L*, a* and b* were lower than in normal meat. The dynamic assessment (within 48 hours) showed increasing pH values. The differences in pH values and their growth dynamics for both types of meat are shown in Fig. 1. The evaluation of the dynamics of colour within 48 hours of measurement is seen in DFD meat reduction of L* (darkening), while the meat was normal to increase L* values (lightening). The course of the dynamics of lightness, both in normal and DFD meat is shown in Fig. 2. Values of

a* were within 48 hours visibly growing only in DFD meat, whereas in normal meat they increased only over 24 hours (0h→24h). The b* values increased in both types of meat only within 24 hours (0h→24h). In normal meat, significant differences in b* were observed in both the 0h→24 and 0→48 periods ($P < 0.05$). Contents of haeme pigments were slightly higher but insignificant in normal meat.

The study showed colour differences, measured by the CIELAB system, in normal and DFD beef. There were noticeable variations in the colour parameters, with the clearance much higher in normal meat, as the value of a* and b*.

ACKNOWLEDGEMENTS

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TRENDS OF CADMIUM CONCENTRATION IN MEAT AND OFFAL OF BOVINE ANIMALS IN THE CZECH REPUBLIC

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ABSTRACT

The study evaluated cadmium (Cd) content in muscle, liver and kidneys of young bovine animals (under two years of age) and cows in the Czech Republic during the period of 1993–2010. We detected significant differences in the average Cd content in cow's kidneys between the years 1993–1994 and 2009–2010 at the level of $\alpha = 0.05$. A significant ($\alpha = 0.05$) increase in Cd content in cow's kidneys between the years 1993–1994 and 2009–2010 was detected as well.

Key words: bovine animals; cadmium (Cd); kidney; trends

INTRODUCTION

Cadmium cumulates in kidney and liver and its half-life of excretion from a human body is long, within an interval of 10–30 years (1). Liver and kidney of food animals can represent an important resource of cadmium due to its accumulation in these organs. The aim of our study was to retrieve and evaluate results of testing for cadmium content in muscle, liver and kidney samples of two age categories of bovine animals in the territory of the Czech Republic during the period of 1993–2010.

MATERIAL AND METHODS

Testing of samples was conducted within the national monitoring of residues and contaminants pursuant to Council Directive 96/23/EC. The minimum number of bovine animals from which samples were taken was of 0.015 % of animals slaughtered in the previous year. Results of testing for cadmium content in muscle, liver and kidney samples of young bovine animals under two years of age except for calves younger than six months (hereinafter referred to as "YB") and cows taken in the territory of the Czech Republic were aggregated by two years for the period of years 1993–2010. Cadmium concentration in the tissues was detected using ETA-AAS and ICP-MS techniques with LOQ of 0.01 $mg.kg^{-1}$ for liver and kidney, and LOQ of 0.005 $mg.kg^{-1}$ for muscle. The evaluation of trends of cadmium content in bovine tissues was performed using statistical testing by comparing two sets of values of cadmium content detected in 1993–1994 with those detected in 2009–2010. The significance of the recorded increasing trend in cadmium values was statistically tested in bovine liver. *F*-test, i. e. the test of the equality of two variances, and *t*-test, i. e. the test of the equality of two means, were used for testing of the two sets of values. The significance of the cadmium content trend in cow's kidneys from 1993 to 2010 was evaluated using statistical testing of the significance of the slope of a line calculated for the trend.

RESULTS AND DISCUSSION

Results included in Table 1 documented that mean values of cadmium in muscles and liver of both groups of bovine animals were in the course of years low, without any apparent trend of an increase or decrease in the values. Cadmium content in cow's liver was, in comparison with cadmium content in YB liver, higher, without any apparent trend of an increase or decrease in both groups of animals. The mean concentra-

Table 1. Cadmium content in bovine tissues in the Czech Republic in the years 1993–2010 (mg.kg^{-1} wet weight)

Tissue	year	Cows (> 2 years) mg.kg^{-1} wet weight					Young bovine (> 6 months < 2 years) mg.kg^{-1} wet weight				
		n	median	mean	10% quantile	90% quantile	n	median	mean	10% quantile	90% quantile
muscle	1993–4	367	0.005	0.008	0.003	0.017	606	0.005	0.008	0.003	0.019
	1995–6	222	0.007	0.008	n. d.	0.013	280	0.007	0.008	n. d.	0.010
	1997–8	152	n. d.	0.007	n. d.	0.014	143	n. d.	0.008	n. d.	0.015
	1999–0	186	n. d.	0.008	n. d.	0.018	167	n. d.	0.008	n. d.	0.010
	2001–2	228	n. d.	0.007	n. d.	0.014	235	n. d.	0.006	n. d.	0.010
	2003–4	180	n. d.	0.010	n. d.	0.015	197	n. d.	0.005	n. d.	0.010
	2005–6	77	n. d.	0.016	n. d.	0.013	74	n. d.	0.004	n. d.	0.013
	2007–8	55	n. d.	0.003	n. d.	0.006	23	n. d.	0.002	n. d.	0.006
	2009–0	49	n. d.	0.002	n. d.	n. d.	31	n. d.	0.002	n. d.	n. d.
		1516		**0.008**			**1756**		**0.007**		
Liver	1993–4	384	0.091	0.111	0.035	0.193	593	0.070	0.085	0.032	0.145
	1995–6	225	0.089	0.111	0.032	0.198	275	0.061	0.071	0.030	0.113
	1997–8	138	0.094	0.109	0.039	0.176	139	0.071	0.081	0.030	0.130
	1999–0	185	0.090	0.127	0.047	0.201	159	0.064	0.079	0.031	0.127
	2001–2	229	0.080	0.099	0.041	0.175	237	0.061	0.074	0.030	0.121
	2003–4	165	0.085	0.099	0.043	0.169	194	0.068	0.074	0.030	0.120
	2005–6	75	0.100	0.122	0.050	0.215	75	0.068	0.079	0.031	0.145
	2007–8	53	0.099	0.116	0.042	0.233	27	0.080	0.081	0.030	0.137
	2009–0	50	0.091	0.119	0.038	0.258	31	0.064	0.079	0.033	0.150
		1504		**0.111**			**1730**		**0.079**		
kidney	1993–4	388	0.380	0.454	0.152	0.860	593	0.210	0.264	0.098	0.509
	1995–6	215	0.397	0.471	0.139	0.862	272	0.206	0.257	0.090	0.497
	1997–8	135	0.421	0.513	0.152	1.039	137	0.260	0.306	0.094	0.616
	1999–0	183	0.457	0.584	0.162	1.095	157	0.227	0.270	0.099	0.464
	2001–2	227	0.432	0.521	0.161	0.990	235	0.207	0.271	0.114	0.475
	2003–4	169	0.434	0.557	0.150	1.072	203	0.230	0.277	0.105	0.477
	2005–6	79	0.520	0.636	0.138	1.340	73	0.271	0.319	0.091	0.606
	2007–8	52	0.619	0.816	0.227	1.862	24	0.200	0.289	0.048	0.789
	2009–0	50	0.588	0.799	0.206	2.110	31	0.226	0.308	0.092	0.823
		1498		**0.533**			**1725**		**0.273**		

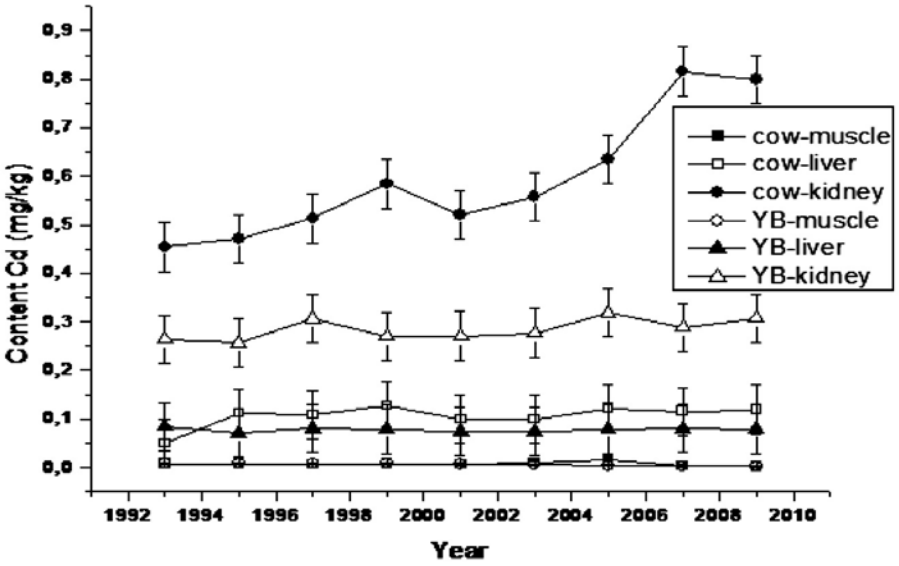

Fig. 1. Trends in the average cadmium content in bovine muscles, liver and kidneys in the Czech Republic between years 1993 and 2010

tion of cadmium in cow's kidney (0.533 mg.kg^{-1}) was twice as high than the average concentration of cadmium in YB kidneys (0.273 mg.kg^{-1}) throughout the monitored period. Similar results were reported by Doganoc who examined cadmium levels in several hundreds of bovine animals for slaughter and detected the following values: 0.004 mg.kg^{-1} in meat, 0.094 mg.kg^{-1} in liver and 0.350 mg.kg^{-1} in kidney (2). Niemi *et al.* (3) detected the following cadmium content: 0.001 mg.kg^{-1} in meat, 0.061 mg.kg^{-1} in liver and 0.350 mg.kg^{-1} in kidney (3). Smith *et al.* (5) detected eight to ten times higher concentrations of Cd in kidney than in liver of cows to which Cd at the dose of 0.025 and 0.125 mg.kg^{-1} b.w. was administered. No difference was recorded in meat of control and experimental animals which indicates that there is no significant affinity of Cd to muscle tissue (5).

As opposed to cadmium content in YB kidney, where there was no apparent tendency to an increase in cadmium concentration in the course of monitored years, the trend of an increase of cadmium content in cow's kidney was proven in the Czech Republic (Fig. 1). The increase in cadmium concentration in cow's kidney was demonstrated by statistical testing at the level of significance of $\alpha = 0.05$. The increase in cadmium concentration in cow's kidney is related to the age of animals as documented e.g. by Pechová *et al.* who confirmed highly significant relationship between the age of cows and cadmium content in kidneys (4). The difference between the mean cadmium content in cow's liver in years 1993–1994 and 2009–2010 detected in our study was significant ($\alpha = 0.05$); the slope of a regression line expressing an increasing trend was significant as well ($\alpha = 0.05$).

CONCLUSION

Statistical evaluation of data confirmed in two independent tests with the probability of 95 % ($\alpha = 0.05$) a significant increase in cadmium (Cd) content in cow's liver in the Czech Republic in the course of the monitored period (1993–2010). No significant increase in cadmium content in the liver and muscle of cows or in YB kidneys, liver and muscles was recorded in the course of the monitored period from 1993 to 2010.

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MEAT QUALITY OF PIGS EXPOSED TO LOW DOSES OF GAMMA RADIATION

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ABSTRACT

Experimental pigs (30 kg b. w.) exposed to ^{60}Co radiation (whole body dose of 0.5 Gy, 0.98 Gy.h^{-1}) were slaughtered 3 days after irradiation. Parameters of meat quality – pH, colour, drip losses, and lactic acid concentrations, were evaluated in *longissimus lumborum et thoracis* muscle. Only the value of meat colour a^*_{45} detected 45 min post mortem was significantly lower ($\alpha = 0.05$) than that of control specimens. The other parameters corresponded to control values. In case of nuclear incident without any internal contamination and providing that the effective dose of 0.5 Sv was not exceeded, the pigs might be processed for human use by the standard meat technology.

Key words: colour; food safety; ionizing radiation; meat quality

INTRODUCTION

Within the last fifty years the ionizing radiation risk has represented a side effect of both testing nuclear weapons and highly unpredictable consequences of nuclear disasters (Chernobyl 1986, Fukushima 2011).

In the 1960s, veterinary radiobiological research was especially focused on the deterministic effects of high doses of ionizing radiation at farm and experimental animals. At the present, great attention has been devoted to the study of low-level doses of ionizing radiation. An imaginary interface between stochastic and deterministic effects of ionizing radiation of human and pig is about 500 mGy. At the present days, the internal contamination of animals by radionuclides can be significantly limited due to modern technologies of pig farming. Pigs can be exposed to the external doses in the animal farms which are located close to a crashed nuclear power plant and/or during their evacuation outside the contaminated areas. However, there is still a question if radiation stress of low-level ionizing radiation doses affects negatively the pork quality.

MATERIAL AND METHODS

Twenty pieces of Slovak Large White pigs (30 kg mean body weight) were used. They were housed at the university (UVMP in Košice) farm in Zemplínska Teplica. Unlike the control group (n = 10) ten experimental pigs were exposed to the ^{60}Co gamma radiation at a whole-body dose of 0.5 Gy with a dose rate of 0.98 Gy h^{-1}. The pigs got used to transport in trucks on the farm some days before transport. They were killed three days after radiation exposure in the slaughter house in Zemplinská Teplica after a short transport and 3 h rest. After the slaughter, samples were taken from musculus *longissimus lumborum et thoracis* at the level of the last rib.

The pH-value was determined within 45 minutes and 24 hours post mortem by means of an Orion 250 A+ digital pH-meter with connected puncture electrode (Monokrystaly Company). The calibration was done by three buffers (4.01; 7.00; 9.00). The pH was determined after stabilization of values automatically diagnosed by the measuring instrument.

Meat colour was determined within 45 minutes and 24 hours post mortem by the spectrophotometric method by a CIELAB system by means of a portable Color Guide Sphere Spex photo color-

imeter (BYK Gardner Company) with elimination of gloss. The resulting colour of slice surface perpendicular to meat fibre direction was represented by an average from three scanned values automatically taken by the photocolorimeter. The results were expressed in the international colorimetric standards CIE L*a*b* (1). The calibration was based on the black L* = 0 and the white L* = 100 (2).

Drip losses were determined by both the standard method according to Popelka *et al.* (4) within the interval from 24 to 48 hours post mortem and by the post mortem method from 0 to 24 hours using beakers (3). Lactic acid concentration was determined according to Popelka *et al.* (4).

The results were processed statistically by means of two-sided t-test with unequal variances. Test criteria were determined at the level of significance of $\alpha = 0.05$.

RESULTS AND DISCUSSION

No statistically significant differences were observed between the experimental and control groups in the observed meat quality parameters of $pH_{45,}$ $pH_{24,}$ $L^*_{45,}$ $b^*_{45,}$ $L^*_{24,}$ $a^*_{24,}$ $b^*_{24,}$ drip losses in the intervals from 0 to 24 hours and from 24 to 48 hours and in lactic acid concentration. The meat colour parameter a^*_{45} determined 45 minutes post mortem was the only exception. The value was significantly ($\alpha = 0.05$) lower (–1.01) in the exposed group compared to the control group (–0.87), the meat was less red. On the other hand, non-significantly higher parameter a^*_{24} was observed in the exposed group of pigs within 24 hours post mortem.

Considering the method used it was interesting that drip losses were identical in practical terms in both observed intervals from 0 to 24 hours (2.00 %) and from 24 to 48 hours (2.03 %). It can be stated that no effect of a dose of 0.5 Gy on pork quality was determined by our test methods.

Based on the well known doses to which the workers were exposed in the vicinity of the Chernobyl nuclear power plant it can be assumed that in emergency planning zones round nuclear facilities the whole-body effective dose of 0.5 Sv would not be exceeded in pigs housed in modern technological facilities. However, there was a prerequisite – to eliminate internal contamination of pigs. This can be achieved by preventing dust penetration into the buildings. Fodder and water contamination should be eliminated in the modern technological facilities by observation of animal hygiene standards

CONCLUSION

The experimental application of a single-use whole-body exposure to a dose of 0.5 Gy did not affect pig meat quality. Pigs can be slaughtered and used for further food applications in case of nuclear facility accidents if no internal contamination occurred and the effective dose of 0.5 Sv was not exceeded. In such cases the standard technological processing of pork products is not limited.

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INFLUENCE OF ADDITIVES AND SOME SUPPLEMENTS ON THE MEAT PROTEIN AND MEAT CONTENT IN MEAT PRODucts

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ABSTRACT

Having tracked ingredients and additives in meat products, their direct impact on the meat protein value and meat content has been confirmed. Meat content defined by the national legislation as the parameter of "minimal meat content in % of weight" was established by an indirect method, based on evaluating the total protein and protein nitrogen as meat indicators. Having used the indirect methods, the indirect influence of ingredients and additives was confirmed, too, had not all the analyses been performed that would have verified their presence, type and amount in a product, and had an inappropriate nitrogen factor been used while calculating the analytical values obtained. In such cases, the latter procedure is likely to let each non-meat ingredient, bearing protein nitrogen, increase the value of meat protein, and the meat content value results accordingly. It has been shown that an objectively chosen parameter plays a major role when the meat protein in meat products is to be directly evaluated.

Key words: additives; meat products; meat protein

INTRODUCTION

With regard to the quality of meat products (MP) produced in Slovakia before 1989, experts evaluated them as having a dominant meat part in comparison to foreign standards, yet having a higher fat content (8). Development should show if consumers continue in preferring these MP, or if they go along the lines of Wellness towards the "lean" products as in Western Europe and the U.S. After 1989, import of MP to our market started and consumers started preferring the new products to the home ones losing a fair judgment towards the original products. Those original products, under the influence of economic changes and pressures, varied by substituting of raw meat in their composition and consequently also in the basic characteristics. Despite the declining quality, MP are still considered expensive and the manufacturer, in an attempt not to produce too expensively, continuously searches for cheaper raw materials; to the extent that the main raw material meat is being alternated or completely replaced by functional additives, substitutes or additives. These may be presented directly in the MP as an ingredient in the form of animal non meat protein (MSM, blood and blood plasma, etc.), vegetable protein (usually soy, etc.), or as a transfer from the ingredients. Resulting from the use of a wider range of non meat ingredients and substituent their detection and quantification in MP using available methods is often rather difficult. Wherever their presence is known (labelled/ from the specification), or specifically monitored, it is possible to demonstrate and evaluate their direct impact (4). In case of not stating the ingredients (in the declaration) the value of meat protein and meat content in the MP may be indirectly affected due to methodology. The choice of indirect methods in the absence of information about the product (especially composition) substantially burdens the results by indirect determinations and additional calculations. In practice, the most common methods analyze substances of total protein nature and also provide determination of all nitrogenous compounds, which delivers results that are biased in favour of the manufacturer.

MATERIALS AND METHODS

We studied the effect of additives and non meat substitutes on the value of meat protein, content of meat, and meat content without fat in soft meat products of Slovakian production. The meat content without fat (MCWF), defined in terms of the Order No. 1895/2004-100 as a parameter "minimal meat content in % of weight" that should be "cleansed" from non meat ingredients, was determined by the methodology of Stubbs and More (9). This analytical approach of indirect methods is based on the principle of determining total protein and protein nitrogen, followed by calculating of the amount of meat in the meat products (5, 6). For a realistic assessment, the impact of non meat ingredients on parameter of content of meat was analyzed and evaluated, deliberately using soft meat products from a group of selected meat products established in the Order No. 1895/2004-100 ("Špekáčky", "Spišské sausages", "Bratislava sausages", "Liptovská salami", etc.), since these can be compared and evaluated against the existing limit.

Knowledge of the indirect effect of additives on the calculated value MCWF, when using indirect methods, was obtained by an alternative calculation with two nitrogen factors (NF): Universal "Kjeldahl" factor N = 6.25 (1, 3) and nitrogen factor for soy Ns = 5.75 (3, 7).

For statistical evaluation of results, linear regression was used in order to verify whether any of the quality parameters indicate the presence of additives in the sample and paired t-test evaluating the difference in mean values of MCWF calculated with and without addition of additives. Evaluation of t-test was used to demonstrate the impact of NF in the calculation on the value of MCWF in meat products.

RESULTS AND DISCUSSION

On the set of 356 samples, the direct impact of ingredients showed notably in soft meat products, particularly in the sausage type. After the analysis focused on quality indicators reflecting total protein (TP), fat and water content, when the ingredients have not been analytically monitored, "Spišské sausages" containing TP varied from 10 to 17 % while MCWF values ranged from 51 to 102 %. "Bratislava sausages" containing TP from 8 to 18 % and the MCWF values were in the range of 34 to 102 % with an alarming 68 % extent of values, which pointed to a serious violation of the meat products quality. Thus, they were suspected for presence of non meat additives and substitutes. Once the most common ingredient in the meat products, soy protein (SoP), was included into analysis, statistical evaluation of samples showed a significant difference of final results of MCWF in situations where the content of soy was not taken into consideration and when it was. Significant difference ($P < 0.001$) between MCWF was calculated without information on the soy content. A direct impact on the value of TP, meat proteins, and meat content in meat products was confirmed. Within three years of soy content monitoring, in the first year the average content was 0.3 % and in the third year the highest value of 5.24 % was determined. A direct impact of substitutes on meat protein and expression of the meat has been demonstrated in a group of samples "Liptovská salami", where there was demonstrated massive falsification using ingredients, which indeed form the character of a product. Mechanically separated meat (MSM), which was declared on the product, was considered by the definition as a component substituting meat. In the sample, in which MSM was declared the only "meat" component, the TP content was 14.69 %, the content of soy 1.37 %, and when reducing soy, the value of MCWT stood at 60.35 %. Wherever the MSM is added into the meat products, its separation and quantification from the value MCWF is not possible. Indirect methods cannot detect and quantify the presence of MSM.

Indirect methods on the principle of determining TP and nitrogen as an indicator of meat in the MP have an important role in analyses used for calculation of meat content. It is necessary to take into account every single ingredient, including meat, embodied by the nitrogen into the MP; and different nitrogen conversion factor (NF) must be used. Lack of detection, due to missing information on the presence of non meat ingredient and the use of an incorrect NF in calculations, has an impact on the value of meat nitrogen, meat protein and the resulting MCWF. When an alternative calculation using the universal nitrogen factor (N = 6.25) instead of calculating the factor of soy nitrogen (Ns = 5.75) is used for the soy detected in the sample, the MCWF is influenced in the same type of MP upward from 0.35 % to 1.98 %. Using an incorrect NF for the ingredients in meat products influenced the MCWF value significantly ($P < 0.05$). This principle equally applies to all ingredients and substitutes introducing the amino-nitrogen into a product.

CONCLUSION

In verifying the quality of meat products is meat with precisely chosen indicators, chemically defined as pure muscle protein (PMP). Knowledge of monitoring the impact of ingredients and substitutes for meat protein and meat content in the meat products was considered important to choose an objective parameter for monitoring the quality of the meat products. Parameter MCWF using the principle of total protein minus collagen adding non meat protein = PMP, appears to be not always objective. Knowledge in its verification pointed out the pitfalls of methodology built on indirect methods, its analytical uncertainty and only the possibility of complicated calculation, thereby any components replacing meat can be considered as meat.

One of the alternatives could be the direct determination and quantification of meat using direct methods for determination of meat indicators: creatinine as muscle protein and 3-methylhistidine as pure muscle protein. This approach would be helpful in elimination of laborious and not always precise monitoring if the effects of additives and substitutes for meat protein on value of meat content and meat protein had a chance to be verified directly.

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NEW SCREENING TESTS DEVELOPED FOR THE DETECTION OF ANTIBIOTIC RESIDUES IN THE MEAT AND KIDNEYS OF FOOD-PRODUCING ANIMALS

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ABSTRACT

Antibiotics are used to treat diseases of both humans and animals. Some antibiotics are also used in the rearing of food animals as a part of breeding programs. However, their use is associated with a risk of the presence of antibiotic residues in the edible tissues of food-producing animals. To protect the health of consumers, the maximum residue limit (MRL) should be established for each antibiotic and each food commodity. Microbial inhibition tests (MIT) are used for controlling the antibiotic residues in foods. The Nouws Antibiotic Test (NAT) and Total antibiotics are the new MIT developed for the detection of antimicrobial residues in slaughter animals and for the detection of antibiotics in milk and meat, respectively. This study presents a short description of both MIT mentioned above.

Key words: antibiotics; microbial inhibition test; residue

INTRODUCTION

Control of residues of veterinary drugs in live animals and animal products has legislative coverage by the Act of National Council of the Slovak Republic No. 39/2007 (1) the veterinary health care and government regulation of the Slovak Republic 320/2003 (5) the monitoring of certain substances and residues in live animals and animal products, as amended. To protect the public health the maximum residue limits of veterinary medicinal products in foodstuffs of animal origin are set by the Commission Regulation (EU) No. 37/2010 (3).

The term "maximum residue limit" (MRL) means the maximum residue limits of pharmacologically active substances which may be permitted in foodstuffs of animal origin. Determination of MRL shall be in accordance with generally accepted principles of safety assessment of the substances. Monitoring subjects are animals, from which the animal products are obtained, their feed and water and products of animal origin, too.

Control of residues of veterinary drugs in live animals and foodstuffs of animal origin must be carried out by using methods validated according to Commission Decision 2002/657/EC (2). The competent authorities approve following official methods of laboratory diagnosis of food for initial screening residues. The first one is plate method – a screening test for antibiotic residues using five bacterial strains (STAR) and the second are methods in vials: PREMI®TEST, KALIDOS TB, MP and ECLIPSE 50.

NEW METHODS OF ANTIMICROBIALS RESIDUES SCREENING

The Nouws Antibiotic Test (NAT)

This method was developed by Pikkemaat *et al.* (6) as an effective alternative to NDKT (New Dutch Kidney Test). NDKT was developed for detection residues of antimicrobials in food animal tissues by the authors Nouws *et al.* (4). It is a simple plate system

using *Bacillus subtilis* BGA as a test strain. This test is based on the analysis of renal pelvis' fluid by paper discs. The NAT-screening combines a simple and efficient sampling and sample processing strategy with a high detection capacity. NAT, as well as NDKT, is based on the analysis of fluid from the renal pelvis. This method is extended from the one-plate-test system to five-test-test system in comparison with NDKT to ensure effective detection of nearly all veterinary medicinal products at the MRL in the kidney. Another benefit of the method consists in identifying a group of antibiotics in suspected samples, which facilitates the subsequent post-screening or chemical confirmation. The system detects tetracyclines, beta-lactams, macrolides, quinolones and sulphonamides.

The principle of the test consists in the growth inhibition of test strain in the presence of inhibitory substance (residue). The test consist of five test plates: *Bacillus cereus* ATCC 11778 for detection of tetracyclines (T-plate), *Kocuria rhizophila* ATCC 9341 for detection of beta-lactams and macrolides (B & M-plate), *Yersinia ruckeri* NCIM 13282 for detection of quinolones (Q-plate), *Bacillus subtilis* BGA for detection of aminoglycosides (A-plate) and *Bacillus pumilus* CN 607 for detection of sulphonamides (S-plate). The sample of renal pelvis' fluid is applied into the holes (diameter 14 mm) prepared in solidifying nutrient medium by sterile cork. Then the plates are incubated at 30 °C (*Bacillus subtilis* ATCC 11778 and *Yersinia ruckeri* NCIM 13282) and 37 °C (*Bacillus pumilus* CN 607, *Kocuria rhizophila* ATCC 9341, *Bacillus subtilis* BGA) for 16–18 hours. Samples should be taken always from fresh kidneys. Five filter paper discs are used for sampling. Their diameter is 12.7 mm and their absorption capacity is 100 µl of tissue fluid. Discs are inserted into the sliced renal pelvis. The results are evaluated according to the average sizes of inhibition zone which have formed around the holes with the examined samples. Samples are considered positive if the average size of inhibitory zones of ≥ 15 mm. When there is a positive result on the one of the plates, post-microbial screening is carried out using meat juice (NAT-meat) or fluid from the renal pelvis (NAT-kidney) as follows (7).

NAT-meat

Post-screening is carried out at the plate, which shows a positive result for NAT. The plates are further optimized for specific substances of investigated matrix. Supernatant, which was obtained by centrifugation an inactivated homogenized muscle, is used for the analysis. Mixing the sample with Tris buffer and bromelain protease is required before the centrifugation in the detection of aminoglycosides' residues. NAT meat comprises five test plates, like NAT. B&M plate (*K. rhizophila* ATCC 9341) detects beta-lactams and macrolides and is supplemented with 0.2 % Tween 20 and tylosin (0.0075 µg.ml^{-1}). T-plate (*B. cereus* ATCC 11778) detects tetracyclines and is supplemented with chloramphenicol 0.5 µg.ml^{-1}. S-plate (*B. pumilus* CN 607) detects of sulphonamides and is supplemented with trimetoprim (0.007 µg.ml^{-1}). Q-plate (*Y. ruckeri* NCIM 13282) for detection of quinolones is supplemented by a 1 M phosphate buffer pH 6.5 in volume of 5 %. A-plate (*B. subtilis* BGA) for detection of aminoglycosides is without supplements. Investigated samples are applied in amount of 250 µl (300 µl for S-plate) to the prepared holes in the nutrient medium. Specific synergistic substances and phosphate buffers are added to the holes with sample further. Samples are considered to be positive if the average size of inhibition zones around the holes with tested samples is ≥ 15 mm.

NAT-kidney

In this procedure, unlike NAT, sample is supernatant, which is obtained by centrifugation of homogenized kidney samples. NAT-kidney consists of four test plates. In the comparison with the NAT-meat, NAT-kidney doesn't use A-plate with *B. subtilis* BGA, but *B. pumilus* CN 607. S-plate is completely missing. Only B & M-plate (*K. rhizophila* ATCC 9341), which detects beta-lactams and macrolides is supplemented with Tween 20 (0.2 %) and tylosin (0.0075 µg.ml^{-1}). T-plate, Q-plate and A-plate are identical with the NAT test. Investigated samples are applied to the prepared holes in the nutrient medium in amount of 250 µl. Specific synergistic substances and phosphate buffers are further added to the holes with tested samples of B & M, Q and A-plate. Any addition of specific substances is not required in the T-plate. Samples are considered to be positive if the average size of inhibition zones around the holes with the tested samples is ≥ 15 mm. There is one exception, aminoglycosides have the size of inhibition zones ≥ 20 mm.

Total antibiotics

Total antibiotics is the test, which was developed for the rapid detection of antibiotic residues in milk and meat of food animals by Euroclone S. p. A. (Italy). This method involves the tubes (TB) and microplate (MT) format. It is a suitable screening test in the laboratory application and food industry due to its simplicity and short time limits and multi residues. The principle of the test is in the growth inhibition of test strain Bacillus stearothermophilus var. calidolactis, which is presented in the agar medium. The test procedure has these steps: the muscle samples are mixed with 10 ml of extraction buffer, which is diluted 1 : 10 (v/v) with distilled water. After mixing, the samples are left at room temperature overnight or they are incubated at 37 °C for 2 hours. The clear supernatant is used for testing. Samples are incubated at 65 °C for 2.5 to 3 hours. Colour of agar changes from violet to yellow in the case of a positive sample. Colour of the agar remains unchanged (violet) in the negative samples (8).

CONCLUSION

Currently Total antibiotics and NAT do not belong among the official methods of laboratory diagnostics of food intended for screening residues of antimicrobial agents, but they represent new alternatives to currently used microbial inhibition tests. The advantages of these tests are their simplicity, multi-residues and higher efficiency of identification of antibiotic residues in food matrices during the screening residues process.

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EFFECT OF SUPPLEMENTATION OF PROBIOTICS TO PIGLETS ON THE RIPENING OF THEIR MEAT

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ABSTRACT

We evaluated the ripening of piglet's meat after supplementation of their feed with probiotics. Piglets in the control group C were fed only mixed feed, G1 group received probiotics (*L. plantarum*, *L. fermentum*) in the form probiotic cheese (4 g/1 piece/day), G2 group was supplied feed with linseed and probiotics. Lactic acid, phosphates and pH of back and thigh samples were measured on days 1, 3 and 11 after killing and storage at 4 °C. Lactic acid and phosphates were higher and pH values lower in back muscles in C and G1. Probiotics caused that decrease in lactic acid, phosphates and increase in pH were not recorded in G1. The combination of probiotics and linseed caused increase in lactic acid, phosphates and decrease in pH in G2.

Key words: lactic acid; pH of meat; phosphates; piglets; probiotics

INTRODUCTION

Production of meat depends on morphological and physiological properties of an organism, various factors of external environment and animal nutrition (1, 5). Probiotics are live microbial feed supplements which in sufficient quantity beneficially affects the host. Probiotics and bioactive substances of natural origin have favourable influence on formation of the gastrointestinal ecosystem (4, 7). Lactobacilli produce lactic acid. It affects pH of the intestine which suppresses pathogens and inhibits their multiplication. Probiotics can be supported by polyunsaturated fatty acids (PUFA). These essential acids (e.g. linseed, which is rich source of α-linolenic acid) get to the digestive tract with feed (8). They affect the growth or adherence of bacteria, influence composition of microflora and metabolic parameters of piglets (3).

The aim of this study was to evaluate the effect of probiotics alone and in combination with PUFA on the ripening process of piglet's meat.

MATERIAL AND METHODS

The experiment was conducted on 24 piglets, 14 daysold. The control group received commercial feed mixture. In Group 1, the feed was supplemented with probiotic strains (L81 – *Lactobacillus plantarum* and 2I3 – *Lactobacillus fermentum*) in the form of probiotic cheese (4g/1piece/day). Group 2 received probiotics and feed mixture (OŠ-02 NORM TYP, SR) enriched withby linseed (Flanders) with 10 % α-linolenic acid. The piglets were killed by bleeding on the 28th day of life. Thigh and back muscles were kept in a refrigerator at 4 °C. The pH level was measured in meat and water extract of meat by a pH meter (WTW-525) and lactic acid and phosphates by an electrophoretic analyser, EA-102 (Villa Labeco, SR) on days 1, 3 and 11 of the experiment.

RESULTS AND DISCUSSION

Conversion of meat muscles is an energy-intensive process related to decomposition from ATP to ADP and inorganic phosphate and, at the same time, degradation of glycogen (2). The formation of lactic acid, phosphates and decrease in pH in the control group were in the range of physiological values for fresh meat throughout the experiment.

Table 1. Lactic acid, phosphates (g.100g^{-1}) pH in meat and extract of meat on days 1, 3 and 11 of the ripening process in chops and thigh of piglets

Day of meat ripening	C L.A.	C PO_4	C pH 1	C pH 2	G1 L.A.	G1 PO_4	G1 pH 1	G1 pH2	G2 L.A.	G2 PO_4	G2 pH 1	G2 pH 2
Back 1	1.26	0.46	5.83	5.99	1.33	0.45	5.95	5.60	1.30	0.39	5.52	5.38
Back 3	1.16	0.44	5.96	5.98	1.19	0.46	5.52	5.55	1.62	0.51	5.79	5.52
Back 11	1.03	0.52	6.15	6.29	1.35	0.49	5.71	5.19	1.80	0.49	5.86	5.50
Thigh 1.	1.02	0.41	6.04	6.08	1.13	0.44	5.98	5.77	1.23	0.41	5.50	5.48
Thigh 3.	1.16	0.42	6.12	5.80	0.97	0.48	5.74	5.81	1.81	0.50	5.75	5.30
Thigh 11.	1.06	0.47	6.01	6.01	0.97	0.47	5.87	5.60	1.81	0.49	5.85	5.45

L.A – lactic acid; pH 1 – meat; pH 2 – meat extract; C – control; G – group; Sk1 – Probiotic; Sk2 – Probiotic/Linseed

A significant decrease in of lactic acid in the meat of piglets was not observed in Group 1 after 11 days of supplementation of probiotics. The levels of phosphates increased and pH level decreased. Increased levels of lactic acid and phosphates and lower pH were found in back compared to thigh muscles. The final pH of meat is affected not only by lactic acid but also by phosphates and other ions (6).

The addition of probiotics and linseed caused the most important production of lactic acid and a slightly higher levels of phosphates and decreased pH in Group 2. Concentrations of lactic acid and pH levels in the thigh and back muscles of piglets were similar.

CONCLUSION

Probiotics and PUFA synergistically increased production of lactic acid as well as phosphates, an important component of energy metabolism, and influenced pH of piglet meat. New form of application of probiotics was tested (maturing hard cheese). The advantage is its acceptable taste for piglets, health safety, low price and stabilization of the active substance up to 6 months. All this ensures maximum efficiency of probiotics at the time of application.

ACKNOWLEDGEMENTS

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LINSEED ADDITION TO FEEDS FOR PIGLETS AND ITS INFLUENCE ON MEAT RIPENNING PROCESS

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ABSTRACT

The study investigated changes in the concentrations of lactic acid, phosphates, and pH values of muscles of pigs fed with feedstuff supplemented with linseed during meat ripening process in comparison with the control group. The results showed that the added linseed with a higher content of α-linolenic acid influenced production of lactic acid in muscles. Lactic acid level was higher in muscles of the experimental group throughout the maturation. Differences in pH were less pronounced with lower pH in muscles of the experimental group. Concentrations of phosphates were similar in both groups. According to these results the shelf life of the meat obtained from pigs fed with feedstuff supplemented with linseed was extended. However, this assumption has to be confirmed by the microbiological examination.

Key words: linseed; piglets; ripening process

INTRODUCTION

Pork is the most consumed meat in the world (1). Meat is one of the main sources of fat in the diet of humans, whose importance lies in the presence of polyunsaturated fatty acids (PUFA). PUFA have an irreplaceable role in the organism as precursors of the amount of biologically active substances.

Scientists involved in human nutrition focused in recent years on polyunsaturated fatty acids and the mutual relationship between omega-6 and omega-3 fatty acids (4). The recommended ratio of n-6/n-3 PUFA is less than 4 (3, 5). Increasing awareness of the consumption of foods with higher content of n-3 PUFA led to the adjustment of the fat component of meat through a modified diet of slaughter animals.

The aim of our study was to monitor the selected parameters (pH, concentration of lactic acid and phosphate) during the ripening process of meat from pigs fed with rations enriched with linseed.

MATERIALS AND METHODS

Pigs at the age of 14 days (n = 18) were used in this experiment. Piglets were divided into two groups (9 + 9) and fed as follows: animals from the first group (experimental) were supplied commercial mixed feed IP-02 TYPE NORM (Spišské Vlachy, SR) enriched with linseed Flanders with a high proportion of α-linolenic acid (10 % of dose); second group of pigs (control) was fed with non-supplemented commercial mixed feed.

On day 28 of age the pigs were killed by bleeding after stunning under veterinary supervision, in accordance with relevant legislation. After slaughtering samples were obtained from thigh and back muscles and stored at 4 °C for 11 days. Subsequently, within 24 hours after slaughter and on days 3 and 11, the muscle tissues were examined. The pH was determined by a probe in the meat and in meat extract and the content of lactic acid and phosphates was determined by an Electrophoretic analyzer EA 102 (VILLA La-

Table 1. Values of pH, concentrations of lactic acid and phosphates (g.100g^{-1}) measured in the samples of back and thigh muscle of the control and experimental pigs

Day	Parameters	Control group		Experimental group	
		Back muscle	Thigh muscle	Back muscle	Thigh muscle
1	pH (extract)	5.99	6.08	5.73	5.76
	pH (meat)	5.83	6.04	5.84	5.92
	Lactic acid	1.26	1.03	1.59	1.49
	Phosphates	0.46	0.41	0.50	0.49
3	pH (extract)	5.98	5.80	5.58	5.82
	pH (meat)	5.96	6.12	5.97	6.08
	Lactic acid	1.16	1.16	1.53	1.35
	Phosphates	0.44	0.42	0.46	0.47
11	pH (extract)	6.29	6.08	6.02	6.18
	pH (meat)	6.15	6.01	6.07	6.07
	Lactic acid	1.03	1.06	1.26	1.15
	Phosphates	0.52	0.47	0.46	0.40

beco, SR). The levels of lactic acid and phosphates were expressed as means in g.100g^{-1} of sample.

RESULTS AND DISCUSSION

Supplementation of pig feed with linseed had an impact on the composition and properties of fats (2, 6). As shown in Table 1, this adjustment of the feed affected also pH and concentration of lactic acid and phosphates in the muscles of pigs during ripening of meat.

The pH values measured within 24 hours by the probe in the back and thigh muscle of the experimental group were very similar to those measured in the meat of the control group. On the other hand, the pH values measured in the extract prepared from samples of back and thigh muscles of the experimental group were lower compared with the control group. These values reflect the amount of lactic acid measured in muscle samples. Lactic acid concentration was higher in the back and thigh of the experimental pigs.

After three days of refrigerated storage, pH of thigh muscle of experimental animals was similar to the pH measured in the control group but lactic acid concentration was higher. The pH and lactic acid in samples of back muscles differed between the groups. Lactic acid concentration was higher in the experimental group and the pH was higher in control samples.

Even on day 11 of the ripening process the lactic acid concentration was higher in muscles of experimental pigs. The pH level was similar in both groups.

Concentrations of phosphates during the ripening process showed no significant changes and were similar in both groups.

CONCLUSIONS

The addition of linseed to feedstuff of experimental pigs increased lactic acid concentrations in the back and thigh muscles during the ripening process in comparison with the control group. On the other hand, the pH of the muscle of experimental group was slightly lower. Based on these results we can assume that the addition of linseed to feed could extended the shelf life of meat. This hypothesis should be confirmed by microbiological tests.

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OXIDATION AND BACTERIAL STABILITY OF MEAT PRODUCTS PREPARED FROM RAW MATERIAL TREATED WITH AGRIMONY AND SAGE EXTRACTS

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ABSTRACT

The aim of this study was to investigate the antioxidant and antimicrobial effects of dry, water soluble extracts derived from agrimony (*Agrimonia eupatoria* L.) and sage (*Salvia officinalis* L.), added to heat treated meat products. An antioxidant effect of both extracts was observed and was reflected in better oxidative stability in comparison with the untreated control sample. The most significant antioxidant effect was achieved by sage extract which persisted throughout the period of storage. The highest antibacterial effect was reached with extracts from sage.

Key words: antibacterial effect; lipid oxidation; plant extracts; pork meat

INTRODUCTION

Antioxidants are agents that restrict activity and reduce formation of molecular oxygen, convert oxygen to the less reactive or nonreactive state and limit oxidation in organisms (2). Therefore antioxidants are added to food that could be damaged by oxidation (meat products, vegetable oils and fats) due to higher content of fat and polyunsaturated fatty acids. Very important source of natural antioxidants is plant material, due to components that stop fat oxidation, antimicrobial activity and improve the taste of food (9).

Agrimony (*Agrimonia eupatoria* L.) belongs to the family Rosaceae. It contains tannin, essentials oil, organic acids, saponins and flavone colorants, choline and phytoncids which affect mycobacteria.

Sage (*Salvia officinalis* L.) has a strong antibacterial effect proved in oral cavity and throat diseases, very useful at gingivitis and paradentosis. Sage leaves contain 1.5–2 % of essentials oils (5).

The aim of this study was to observe the effect of commercially prepared, water soluble extracts of agrimony on oxidative stability of fat in heat treated meat products stored for 35 days at 4 °C. The antibacterial activity of herbal extracts was also investigated.

MATERIALS AND METHODS

Commercially prepared dry extracts of agrimony and sage were purchased from Calendula a.s. (Slovakia). Ethanol in 40 and 50 % concentration was used as an extracting agent. Whole herb of agrimony and leaves from sage were used for the extraction. The extract was dissolved in water before producing meat products.

Production and storage of heat treated meat products

Three batches of products from pork were produced. The first batch (Control) was prepared without addition of herbs extracts. The second and third batch (agrimony and sage) were treated with herbs extracts at a dose of 2 g.kg^{-1} pork (fat content 30.8 %). Salted raw meat (salt content 2 %) was mixed together with ice (14 %) for 3 minutes below 11 °C. Subsequently, the mixed meat was filled into impermeable polyamide covers (200–300 g) and heat treated (70 °C, 10 minutes). The samples were then cooled and stored (4 °C, 35 days). Samples for examination were taken on days 1, 28 and 35 of storage.

Evaluation of thiobarbituric acid assay (TBA)

The method of thiobarbituric value (TBA) determination (7) was used for determination of lipid oxidation changes in heat treated meat products, expressing the degree of secondary damage to lipids, contingent upon the oxidation of unsaturated fatty acids. Absorbance was measured spectrophotometrically at 532 nm (Heλios γ v 4.6, Thermo spectronic, UK). The results were quantified as malondialdehyde (MDA) equivalents (mg MDA.g^{-1} sample).

Statistical analysis

All the data were analysed statistically using GraphPad Prism Software, Version 4.00 (GraphPad Prism, 2003). One-way analysis of variance (ANOVA) with the post hoc Tukey's multiple comparison test was used to evaluate statistical significance of differences among the control and experimental groups. The results are given as means and standard error of the mean (SEM). $P < 0.05$ was considered as statistically significant difference.

RESULTS AND DISCUSSION

The results of the determination of TBA, measured in heat treated meat products stored in a refrigerator (4 °C) are shown in Table 1.

On the first day of storage, the levels of observed parameters were generally low in all groups; however, there was a significant difference ($P < 0.05$) between experimental trials and the control group. The higher level of malondialdehyde was recorded in the control group in comparison with groups treated with herb extracts. After the storage of samples a positive antioxidant effect of extracts on oxidative stability of fat (28 and 35 days) was observed. The highest oxidative stability and the lowest level of malondialdehyde were found in sample with sage extract addition ($P < 0.05$). Similar results confirming antioxidant properties of sage and agrimony extracts were also recorded by Giao *et al.* (3) and Lahucky *et al.* (6). According to Karpinska *et al.* (4), addition of sufficient amount of antioxidant before meat destruction prevents adverse organoleptic and antioxidant changes. Based on our results, significantly lower oxidative damage to fat in heat treated meat products was observed after addition of antioxidants. It is important to add the effective antioxidant before mixing the raw meat.

Table 1. Results of TBA determination expressed as malondialdehyde equivalents (mg.MDA.g^{-1} sample)

	Day 1	Day 28	Day 35
Control	0.102 ± 0.017	0.251 ± 0.12	0.440 ± 0.024
Agrimony	0.069 ± 0.011[a]	0.217 ± 0.016[a]	0.302 ± 0.021[a]
Sage	0.062 ± 0.013[a]	0.130 ± 0.013[a]	0.203 ± 0.026[a]

[a] – indicates significant difference between columns compared to the control ($P < 0.05$)

Results of microbial assessment are shown in Table 2. No bacteria from the family Enterobacteriaceae nor coagulase positive staphylococci (expressed as colony forming units – CFU) were recovered from heat treated samples stored for 28 days in a refrigerator. This indicates good hygiene practices and proper heat treatment. With regard to the total count of bacteria (TCB) we observed reduced colony counts after treatment with both herbal extracts in comparison with the control. The best antimicrobial effect and the lowest increase in TCB were recorded in samples treated with sage extract. Sekretar *et al.* (8) and Agaoglu *et al.* (1) also recorded similar antibacterial properties of sage and agrimony extracts.

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Table 2. Results of microbiological assessment

	TCB (CFU.g^{-1})		*Enterobacteriaceae* (CFU.g^{-1})		Coagulase positive staphylococci (CFU.g^{-1})	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
Control	$1.6.10^4$	$2.8.10^4$	$< 1.0.10^1$	$< 1.0.10^1$	$< 1.0.10^2$	$< 1.0.10^2$
Sage	$6.5.10^2$	$1.4.10^4$	$< 1.0.10^1$	$< 1.0.10^1$	$< 1.0.10^2$	$< 1.0.10^2$
Agrimony	$2.4.10^3$	$1.8.10^4$	$< 1.0.10^1$	$< 1.0.10^1$	$< 1.0.10^2$	$< 1.0.10^2$

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COMPARISON OF CHILLING OPERATIONS OF SLAUGHTERED ANIMALS

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ABSTRACT

We evaluated the chilling process of slaughtered animals' carcasses performed. Nine measurements were focused on the temperature and time dependence of carcasses chilling (pork halves – 3, bull – 2, calf – 3, cow – 1). The evaluation of the temperature and time dependence of carcasses chilling was performed by digital thermometer DT 301018 and temperature was recorded in one hour intervals. The temperature measurements showed that the stated temperature (+7 °C) was reached during 16 hours. The time necessary for chilling of calf's, cow's, and bull's meat was significantly different. The chilling of bull's meat lasted 23–24 hours, shorter time was necessary for cow's meat (20–21 hours) and the shortest was required by calf's meat (in two cases 13–14 hours, and in one case 17–18 hours).

Key words: beef; carcass chilling; pork

INTRODUCTION

Most post-mortem chilling processes of livestock carcasses are primarily employed to ensure food safety, maximize shelf-life, and reduce shrinkage with less emphasis on maintaining tenderness and colour factors of the finished product. Whether chilling conditions are being met with regard to regulatory requirements, as part of the critical control point (HACCP) system, or as part best practices, additional factors may be more important than those affecting direct consumer satisfaction with the product (5).

There are many biochemical and structural events that take place in the first 24 h period after the animal is slaughtered and the muscle is converted to meat. This period greatly impacts meat tenderness and muscle colour and in species-specific how the initial cooling process results in positive or negative consequences in meat quality (5). The goal of the chilling of meat is to decrease the temperature in the deepest parts of muscles from the temperature of 39–2 °C as soon as possible while maintaining best its weight. Some losses of the weight are necessary and even desirable because dried surfaces are more resistant to the microbial spoilage (1). Due to the danger of spoilage, meat has to be chilled soon after slaughter (3).

EU legislation requires all fresh meat carcasses to be refrigerated immediately after slaughter and dressing to prevent the growth of pathogenic and spoilage bacteria. Post-mortem inspection must be followed immediately by chilling in the slaughterhouse to ensure a temperature throughout the meat of not more than 3 °C for offal and 7 °C for other meat along a chilling curve that ensures a continuous decrease of the temperature (4).

Due to this requirement, the evaluation of chilling process of slaughtered animals carcasses (pig, bull, calf, and cow) was performed.

MATERIALS NAD METHODS

The monitoring of the chilling process of pig (n = 3), bull (n = 2), calf (n = 3), and cow (n = 1) carcasses after slaughter was performed. The temperature was measured using digital thermometer DT 301018 (TFA, Germany). It was recorded in one hour intervals. The chilling curves were obtained on the basis of temperature data.

RESULTS AND DISCUSSION

The curves of carcasses chilling are shown in Fig. 1 and 2. Cooling of pork halves (≤ +7 °C) lasted maximum 16 hours in all three cases. The time needed for bulls, calves, and cow carcasses was significantly different. The longest sustained cooling of bulls meat was between 23rd and 24st hour, shorter in cows (between 20th and 21st hour) and the cooling of calf carcasses lasted the shortest time (in two cases between 13rd and 14th hour; in one case between 17st and 18st hour).

According to Van der Wal (6), rising of temperature from scalding of pigs must be decreased in carcass. Therefore the carcasses are chilled to a temperature ≥ 7 °C and it is very important for meat shelf-life improvement.

Carr *et al.* (2) evaluated the effects of chilling and trimming on the microbial populations. According to those authors the desire to reduce microbial populations on pork carcasses as a food safety issue and the coming implementation of the hazard analysis critical control points (HACCP) programs warrants the use of trimming and chilling methods as

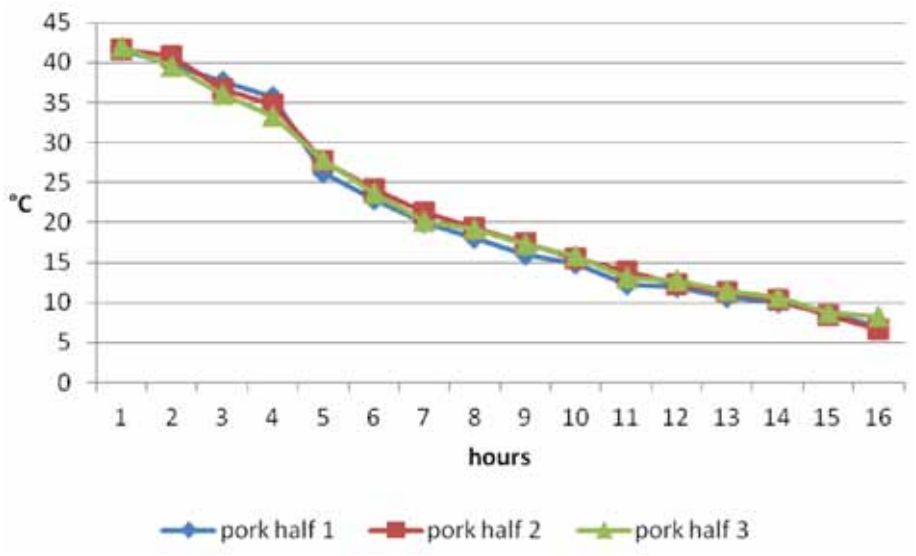

Fig. 1. Comparison of pork carcasses chilling

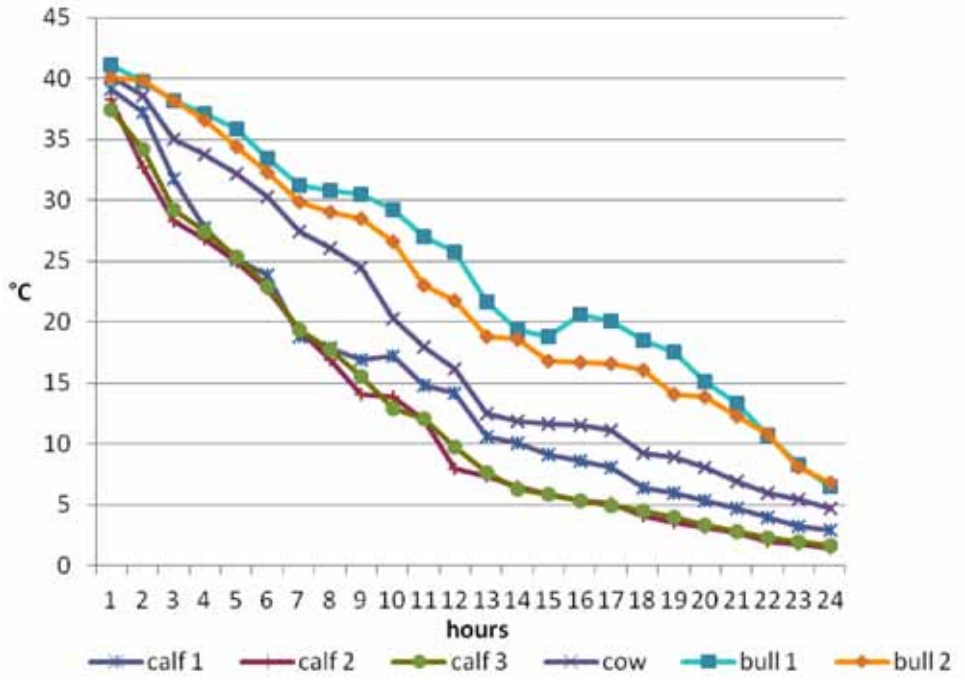

Fig. 2. Comparison of bull, calf, and cow carcasses chilling

critical control points or good manufacturing practices and standard operating procedures in the pork slaughter, processing, and packaging industry.

Biochemical processes and structural changes that occur in muscle during the first 24 hours post-mortem play a great role in the ultimate quality and palatability of meat and are influenced by the chilling process that carcasses are subjected to after slaughter. For beef and lamb, employing chilling parameters that minimize cold shortening is of greatest importance and can be best addressed by ensuring that muscle temperatures are not below 10 °C before pH reaches 6.2. For pork, because of the impact of high muscle temperatures and low pH on the development of pale, soft, and exudative (PSE) pork, a more rapid chilling process is needed to reduce PSE with the recommended internal muscle temperature of 10 °C at 12 hours and 2–4 °C at 24 hours (5).

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HISTOLOGICAL APPEARANCE OF PIGS TESTIS AFTER IMMUNOCASTRATION

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ABSTRACT

The article focuses on the impact of immunocastration on histological appearance of pig testis. The histological appearance was determined at the time of slaughtering. In the group of examined pigs we found histological differences in relation to sexual maturing of pigs. Spermatogenesis up to the phase of spermatide occurred in 67% of pigs, spermatocytogenesis was stopped in the phase of primary spermatocytes in 22%, and proliferation of spermatogonia did not occur in 11%.

Key words: boar taint; immunocastration; spermatogenesis

INTRODUCTION

Castration is implemented particularly due to suppression of boars' sexual maturity which is accompanied by changes in taste and smell of meat (the so-called boar taint). The boar taint is considered to be caused by aromatic heterocyclic organic compounds of skatole, indole and steroid hormone androsterone. Androsterone is already produced in the testes of young pigs in a lesser extent and it reaches peak production during puberty (10).

Immunocastration or a vaccination against boar taint is used in many countries. Today it can be found already in 58 countries of the world (5). This method consists of suppression of hormones responsible for boar taint but also for the development of testes and spermatogenesis. The mechanism consists of blocking gonadotropin releasing hormone (GnRH) in hypothalamic-pituitary-gonadal axis using GnRH antibodies. Immunocastration method seems to be a suitable substitute for surgical castration, which also carries certain surgical risks. This form of castration could also be useful for countries where pigs are not surgically castrated, but in order to prevent the boar odour pigs are slaughtered before the onset of puberty (11). Experiments based on principles other than surgical castration, whether immunological or operative, led to only small success. Experiments based on diet modification which should lead to a reduction of boar taint are worth to mention, however, this effect was not confirmed in practice (6). In another study, reduction of indole and skatole levels due to adjusting the diet was confirmed, but androsterone levels were unchanged (3). Likewise, the effect of seasonality on boar taint was minimal (1).

Another effect is the impact of castration on meat quality of slaughter animals. Differences between surgically and immunologically castrated animals are mainly in the overall dressing percentage, in the amount of intramuscular fat and back muscle thickness (4).

MATERIAL AND METHODS

Sampling

Testes used for detection came from a random set of immunologically castrated pigs. For the purposes of evaluation the whole testes were removed during evisceration in slaughterhouses. For further histological processing another sample was taken from the caudal part of the right testis of the volume of 1 cm^3. Fixation of samples took place over 24 hours in 10% formaldehyde solution.

Microscopic examination

Samples were cut on a rotary microtome of Leica RM2255, the section thickness was 4 µm. Sections were mounted on slides. The microscopic slides were stained by hematoxylin-eosin staining, as

described in the protocols manual for histology of food (Metodika, 2005). Microphotographs of random locations of the investigated samples were prepared for the image analysis using a light microscope of NIKON Eclipse E220. We used 400 magnification. Images were captured by Canon PowerShot A620 and software for image capture of PSRemote, Version 1.6.4. From all the microphotographs captured, 3 random images were selected for each sample (randomization, www.random.org) and these were examined by image microanalysis. These images were examined using ACC software, version 6.1 (SOFO, CZ). Percentage of seminiferous epithelium, lumen of seminiferous tubules and interstitial tissue were determined in the investigated images.

RESULTS AND DISCUSSION

In the microscopic preparations, structure of seminiferous tubules, amount of interstitial tissue, and process of spermatogenesis was observed. In 67 % of the examined samples, spermatogenesis proceeded to the stage of spermatids. In these individuals there were probably also produced sperms capable of fertilizing the female egg. It is therefore clear that the hypothalamic-pituitary-gonadal axis was not completely interrupted. The pigs reached puberty and formation of androstenone causing that the boar taint also occurred (3). In 11 % of the samples proliferation of spermatogonia was not observed. The development of embryonic epithelium stopped at the stage of prepubertal boar. In 22 % of the samples spermatocytogenesis was stopped in the phase of primary spermatocytes. In these samples, therefore, formation of sperm did not occur and boars would not be able to fertilize the female egg. Hormone levels were probably at a lower level than in non-castrated animals. Confirmation of the presence or absence of androsterone would be possible for example from plasma (2) or pig fat (7). The relative representation of the area of stratified epithelium, lumen of seminiferous tubules and connective tissue classified by phase of spermatogenesis (expressed in percentages) is shown in Table 1, including the length of the testes.

In other studies differences within the group immunocastrated animals are also described. Weight of testes of immunocastrated pigs is lower than of not castrated boars by 16 to 60 %, while production of androstenone is below 0.04 $\mu g.g^{-1}$ (8). As stated by Oonk *et al.* (9), androstenone in testes smaller than 9 cm did not exceed the amount of 0.5 $\mu g.g^{-1}$, the amount of testosterone was small in testes smaller than 8 cm. For skatole, testicular size does not correlate with its amount. In both studies, boar taint was not detected in sensory evaluations.

Differences in the development of stratified epithelium also correlate with testes size, and as already described in these studies, these findings are common in the case of immunological castration.

CONCLUSION

Within the group of animals tested, we found histological differences in relation to sexual maturation of pigs. In 67 % of the pigs spermatogenesis progressed to the phase of spermatids formation, in 22 % of pigs spermatogenesis stopped at the phase of primary spermatocyte, and in 11 % of pigs proliferation of spermatogonia was stopped. Testes size correlated well with histological findings.

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Table 1. Comparison of histological structures representation (%) and testis length

Phases of spermatogenesis	Stratified epithelium	Connective tissue	Tubule lumen	Presence of spermatids	Length of testis
Without the proliferation of spermatogonia	37.44	35.62	26.93	unconfirmed	6 cm
Spermatocytogenesis in phase of primary spermatocyte	53.15	24.46	22.36	unconfirmed	12 cm
Spermatogenesis to the phase of spermatids formation	67.97	25.19	6.85	confirmed	12.75 cm

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MOLECULAR IDENTIFICATION AND TYPING OF *STAPHYLOCOCCUS AUREUS* – ENVIRONMENTAL CONTAMINANTS OF MEAT PROCESSING FACILITIES

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ABSTRACT

***Staphylococcus aureus* is considered worldwide as one of the most important human pathogens, its enterotoxin-producing strains being particularly dangerous. The bacterium causes a variety of diseases ranging from skin infections to life-threatening conditions such as pneumonia or endocarditis. Bacteria of this species are frequently detected in food processing facilities from where they can spread to food products and consumers. Characterization of these strains is important for monitoring the spread of infection in the human population. In our study, 323 coagulase-positive Staphylococcus sp. isolates from Slovakian meat- and cheese-processing facilities were analysed, 64 of them being classified as *Staphylococcus aureus* using a real-time polymerase chain reaction (PCR) targeted to acriflavine resistance (acrB) and methyciline resistance (mecA) genes. The strains were further analysed for the presence of four genes responsible for enterotoxin production (sea, seb, sec a sed). Diversity of *Staphylococcus aureus* in food processing environments and food products was studied by another PCR-based method, namely, multiple loci variable number of tandem repeats analysis (VNTR). The combined use of these genotyping methods provided rapid, sensitive and reliable identification and typing of Staphylococcus aureus.**

Key words: PCR; MLVA; *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus are facultative gram-positive bacteria that can occupy various environments including human or animal skin and nasal tissue. It also causes several diseases from the common ones to life-threatening conditions (5). Extensive genetic variability of *S. aureus* is a basis for monitoring of its origin and epidemiology (3). Sequencing of its genome revealed the presence of tandem repetitions with variable number (VNTR) in many loci, e. g. clfA, clfB, sdr, ssp or spa. These genes encode surface proteins, serine protease and protein A, which play a significant role in pathogenicity. Detection of VNTR is possible by PCR. The number of repetitions may differ with strain and amplification of tandem repetitions in these loci may be used for typing *S. aureus* strains (4). The method is important because it facilitates rapid, sensitive and reliable identification and typing of *S. aureus* to monitor its transfer from infected animals to humans via the food industry and to improve the efficiency of hygiene control in the food production.

MATERIAL AND METHODS

Cell-lysate preparation: Cell sediment obtained from 1 ml of the overnight-grown culture of *S. aureus* was suspended in

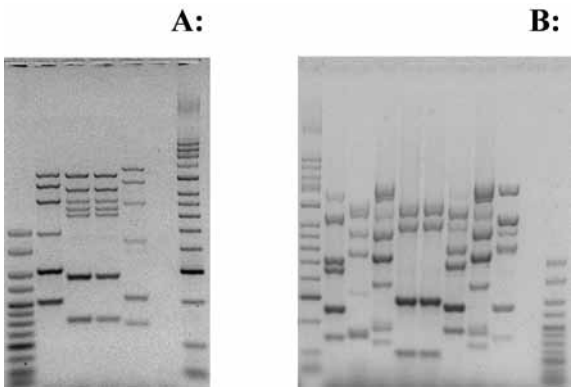

Fig. 1. MLVA profiles of *Staphylococcus aureus* strains
A: Lanes 1 and 7 are standards of molecular weight, lanes 2–5 are *S. aureus* strains: 2 – MSSA1433, 3 – MSSA1253, 4 – MSSA1256, 5 – MRSA1679
B: Lanes 1 and 11 are standards of molecular weight, isolates form food factories: lanes 2–4 are isolates from a cheese-producing factory, lanes 5–8 isolates from a meat-processing factory, lane 9 – SA1679

100 µl lysis solution (20 mmol.l^{-1} Tris HCl pH 8.2 mmol.l^{-1} EDTA, 1.2 % Triton X-100). Lysate was incubated at 95 °C for 20 min and then centrifuged at 13 000 × g for 3 min.

Real-time PCR: A duplex real-time PCR was used to amplify acrB (acriflavin resistance) and mecA (methycillin resistance). The reaction mixture contained 4.5 mmol.l^{-1} $MgCl_2$, 200 mmol.l^{-1} dNTP, 200 nmol.l^{-1} of each probe, 100–300 nmol.l^{-1} of each primer, 1.5 U Cheetah Hot Start Taq DNA polymerase (Biotium, Hayward, USA) 1× buffer supplied with the polymerase and 2 ml DNA template solution. Thermal program 95 °C 2 min; 40 cycles (95 °C 15 s; 60 °C 1 min) was accomplished using the thermocycler iQ5 (Bio-Rad, Hercules, California, USA). Oligonucletides used for acrB detection were aurF (cta gct tta ttt cag cag gtg acg at), aurR (tca aca tct ttc gca tga ttc aac ac), aurP probe (FAM-ctt gct ccg ttt cac cag gct tcg gtg-TAMRA), and for mecA detection mecA-F (cat tga tcg caa cgt tca att t), mecA-R (tgg tct ttc tgc att cct gga), mecA-P probe (JOE-tgg aag tta gat tgg gat cat agc gtc at-BHQ). Fluorescence for FAM and JOE was measured in the corresponding optical channels.

Detection of toxin-encoding genes by real-time PCR: Four simplex PCR were used (2). PCR conditions and the reaction mixture were the same as for duplex real-time PCR described above, but the concentration of each primer was 300 nmol.l^{-1}.

MLVA: In a multiplex PCR (3), the following reaction mixtures were used: 1 µmol.l^{-1} primers for loci clfA, clfB, sdr, 0.5 µmol.l^{-1} primers for loci spa and ssp, 400 mmol^{-1} dNTP and 1.5 U Biotaq DNA Polymerase (BIOLINE) with 1× reaction buffer supplied with the polymerase. Thermal programme was 95 °C 2 min; 20 cycles (95 °C 30 s; 59 °C 30 s, 72 °C 1 min); 72 °C 5 min. The products were separated by 1.75 % agarose gel electrophoresis in TAE buffer at 50 V for 3.5 h.

RESULTS AND DISCUSSION

Samples of swabs and food products were taken quarterly from two food factories, a meat-processing factory and a cheese-producing one. Coagulase-positive strains of the genus Staphylococcus were identified after cultivation and selection on cultivation media according to STN EN ISO 6888. A number of 64 from all 323 isolates was identified as *S. aureus* using duplex real-time PCR on the basis of acrB detection of acriflavine resistance. No strain showed the presence of mecA, a gene responsible for methycillin resistance. We used MLVA PCR according to (3) with a modified mode of typing the confirmed *S. aureus* isolates. We analysed 5 VNTR loci sdr, clfA, clfB, ssp and spa, whereas sdr locus contains three open reading frames sdrC, sdrD and sdrE arranged in tandem. These encode Sdr surface proteins connected with pathogenicity.

In the meat-processing factory, 12 *S. aureus* isolates were detected, which were classified into 3 profile classes differing in the copy number of VNTR loci. In the cheese-producing factory, 52 isolates were detected, which were classified into 3 profile classes. One class persisted throughout the period of sampling. *S. aureus* of one profile class was detected in both factories (Fig. 1 B, lines 4 and 8). Finally, isolates of all classes were tested for the presence of enterotoxin genes (sea, seb, sec a sed) by real-time PCR. However, none was positively detected in these strains. Use of these three identification and genotyping PCR-based methods facilitated a rapid, high sensitive and reliable characterization of *S. aureus* strains without a need for DNA isolation from the sample. Typing with MLVA has a lower discriminatory power compared to pulse field gel electrophoresis (PFGE) but, as it involves automated fragment sizing, produces unambiguous data that can be easily shared between laboratories (1). Our results and experience indicate that these methods may be useful to improve the control of the food production, including the verification of sanitation efficiency.

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BEEF MEAT TEXTURE PARAMETERS INFLUENCED BY CATTLE CATEGORY

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ABSTRACT

The aim of this study was to evaluate texture and chemical parameters (dry matter, fat, collagen and pure protein) in four categories of cattle. Cattle came from a small-scale farm and a large-scale farm. Texture parameters: hardness, cohesiveness and shear force, and chemical parameters were determined in *Musculus gluteus medius*. The category of young bulls from the small-scale farm had the lowest amount of dry matter and fat. Meat from cows from the small farm was less tender than meat from young bulls and bulls. The category of young bulls from the large-scale farm had the lowest amount of dry matter. We did not find statistical differences in hardness TPA between cattle categories. We found statistical differences in texture only between bulls when we compared the scale of farms.

Key words: bulls; cows; chemical composition; heifers; meat; texture

INTRODUCTION

Modern meat production techniques aim to increase the muscle weight and meat quality, but these characteristics are not positively correlated. Different breeds of cattle have a wide spectrum of fibre types in muscles but these are not always reflected by differences in instrumental analyses using Warner Bratzler or sensory panels. Within the same breed type, the most important intrinsic factors affecting meat quality is age, live weight and sex. (3). A number of authors have compared the carcass and meat quality between bulls and heifers. Females yielded a comparable dressing percentage, but carcass fat content was considerably higher. Most references showed that tenderness of beef from females was better than from bulls. It was reported, that tenderness was generally superior for heifers (2). The objective of this study was to evaluate the influence of cattle category and the type of farm (small-scale and large-scale) on beef texture and chemical composition.

MATERIALS AND METHODS

Samples (*Musculus gluteus medius*) were from beef from small-scale farm (10 heifers, average age 22–26 months; 10 cows, average age 41–91 months; 10 young bull, average age 18–23 months; 10 bulls, average age 24–28 months) and from beef from a large-scale farm (13 cows, average age 37–75 months; 13 young bull, average age 16–19 months; 13 bulls, average age 27–30 months). Samples (100 g) were homogenised and the following parameters were subsequently determined: amount of dry matter (sea-sand drying, 24 hours at 103 ± 2 °C, CSN ISO 57 6021), fat (analysed on the SOXTEC instrument made by TECATOR, with diethylether as extraction agent), collagen (spectrophotometry at 550 nm, equalized to 4-hydroxyprolin content) and pure muscle protein (after elimination of non-protein N-compounds by hot tannin, protein content was measured on KJEHLTEC from TECATOR, pure muscle protein was calculated as the protein content reduced by the amount of collagen). Nitrogen was converted to crude protein using the factor of 6.25.

Samples were tested by Texture Profile Analysis (TPA) and by Warner-Bratzler test using Instron 5544 (Instron Corporation, England). For TPA, cylinder samples (1 cm high, 1.25 cm diameter) were compressed twice to 50 % of their original height. Hardness (N) – peak force required for first compression, cohesiveness – ra-

tio of positive force area during the second compression to that in the first compression were evaluated (4). The shear force was measured on samples (1.25 cm wide, 1.25 long, 1 cm high) by Warner-Bratzler (W-B) test. The texture was measured on cooked meat after heat treatment. The meat was heat treated in plastic bags separately in a water bath (72 °C), the core temperature of samples was kept at 70 °C for 90 min. Samples were cooled at room temperature and stored overnight at 4 ± 2 °C (1).

Statistical evaluation of results was conducted using statistic software STATISTICA 7 CZ (StatSoft, Czech Republic).

RESULTS AND DISCUSSION

The small-scale farm (Table 1): The category of young bulls had the lowest amount of dry matter and fat. We found statistical significant differences ($P \leq 0.05$) in amount of dry matter and fat between young bulls and other cattle categories. Meat from female cattle contained less collagen content and more fat content compared with meat from male cattle. We did not find statistical significant differences in hardness TPA between cattle categories. We found statistical differences between heifers and cows in maximum shear force (W-B test), heifers had meat more tender than cows, and this corresponds to Sañudo *et al.* (3). Meat from cows was less tender than meat from young bulls and bulls. Within the same breed type as age, sex and slaughter weight of the animal most important factor influencing meat quality. Meat from animals with a higher slaughter weight is less tender. The meat hardness increases with the age of animals (3).

The large-scale farm (Table 2): The category of young bulls had the lowest amount of dry matter. We found statistical significant differences ($P \leq 0.05$) in amount of dry matter between young bulls and cows. We did not find statistical differences in hardness TPA between cattle categories. We did not found statistical differences between cows and bulls in maximum shear force (W-B test); young bulls had more tender meat than cows and bulls.

Table 1. Quality parameters of beef meat from the small-scale farm

	Heifers	Cows	Young bulls	Bulls
	Mean ± S. D.	Mean ± S. D.	Mean ± S. D.	Mean ± S. D.
Dry matter (%)	28.752 ± 3.40	26.666 ± 2.09	24.970 ± 1.19	26.127 ± 1.16
Fat (%)	4.146 ± 1.77	3.293 ± 2.41	1.483 ± 1.02	2.517 ± 1.73
Collagen (%)	1.006 ± 0.35	0.908 ± 0.22	1.211 ± 0.30	1.342 ± 0.39
Pure protein (%)	19.540 ± 1.67	19.383 ± 1.32	19.361 ± 1.64	19.508 ± 1.29
Hardness TPA (N)	62.035 ± 15.78	67.051 ± 7.57	60.307 ± 18.53	63.413 ± 10.92
Cohesiveness (–)	1.278 ± 0.02	1.282 ± 0.01	1.253 ± 0.02	1.270 ± 0.03
Shear force W-B (N)	74.395 ± 16.63	91.158 ± 10.85	65.797 ± 13.43	69.812 ± 11.52

Table 2. Quality parameters of beef meat from the large-scale farm

	Cows	Young bulls	Bulls
	Mean ± S. D.	Mean ± S. D.	Mean ± S. D.
Dry matter (%)	27.519 ± 1.50	25.672 ± 1.38	26.299 ± 1.69
Fat (%)	4.158 ± 2.97	2.990 ± 2.01	2.306 ± 1.46
Collagen (%)	1.317 ± 0.42	1.218 ± 0.29	1.335 ± 0.35
Pure protein (%)	20.329 ± 1.16	19.522 ± 1.47	19.217 ± 1.39
Hardness TPA (N)	61.485 ± 17.43	65.713 ± 17.86	65.320 ± 22.42
Cohesiveness (–)	1.276 ± 0.01	1.266 ± 0.02	1.309 ± 0.09
Shear force W-B (N)	95.267 ± 21.49	78.416 ± 18.92	89.292 ± 21.19

When we compared the small-scale farm with the large-scale one, we found statistical differences ($P \leq 0.05$) in the amount of fat between young bulls; young bulls from the small-scale farm had less amount of fat. We found statistical differences in texture only between bulls; meat from bulls from the large-scale farm was less tender.

CONCLUSION

The small-scale farm – young bulls had the lowest amount of dry matter and fat, cows was less tender than meat from young bulls and bulls. The large-scale farm – young bulls had the lowest amount of dry matter; we did not find statistical differences in hardness TPA between cattle categories. We found statistical differences ($P \leq 0.05$) in texture only between bulls when we compared the two farms.

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INNOVATIVE APPROACH TO DETERMINATION OF BIOGENIC AMINES IN A MATRIX OF ANIMAL ORIGIN

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ABSTRACT

A rapid ultra-performance liquid chromatographic (UPLC) method for the determination of biogenic amines (histamine, tyramine, phenylethylamine, tryptamine, agmatine, putrescine, cadaverine, spermine, and spermidine) in meat products is described. Nine biogenic amines were derivatized with dansylchloride and separated on a X-Bridge BEH C18 column using gradient elution and UV detection. The analysis was very rapid and all amines were well resolved and eluted from the column in less than 8 min. The average repeatability ranged between 0.77% and 1.27%. The method was applied for the analysis of 19 samples of meat products, as retailed in the Czech Republic. UPLC results were compared to those obtained by a conventional HPLC method.

Key words: biogenic amines; HPLC; meat products; UPLC

INDRODUCTION

Biogenic amines (BAs) are low molecular weight organic bases that possess the biological activity. They are usually produced, with the exception of the physiological polyamines, by the microbial decarboxylation of amino acids. BAs are present in fermented foods as a result of technological process. Their presence in non-fermented foods indicates the spoilage of food by the action of saprobic bacteria. The consumption of food with high BAs concentration can lead to different degree of food intolerance (2). Therefore, it is desirable that BAs in foods are as low as possible.

A large number of methods were developed for the determination of BAs in food. The currently used methods have been reviewed e.g. by Önal (3). BAs are mostly determined by chromatographic techniques, in particular by HPLC with fluorescence or UV/DAD detection (3). Ultra-performance liquid chromatography (UPLC) not only maintains the resolution and efficiency of HPLC, but also significantly reduces analysis time and the consumption of solvents used as the mobile phase (1). Chromatographic columns with sub-2μm particles and high back-pressure are the main characteristics of this method.

The aim of our study was to convert the HPLC method (4) to the UPLC method, to determine BAs in various meat products and to compare the results.

MATERIALS AND METHODS

Samples

Samples (n = 19) of heat-treated and fermented meat products were collected from the retail shops in South Moravia, the Czech Republic.

Reagents and apparatus

Biogenic amine standards (mostly as hydrochloride salts) and other chemicals were of the highest purity available. BAs were supplied by Sigma Aldrich (USA), other chemicals were purchased from Merck, (Germany). Samples were homogenized with Ultra-Turrax T 25 homogenizer (Ika, Germany). Other instruments: rotary vacuum evaporator (Büchi, Swiss), laboratory shaker, water bath.

UPLC conditions

UPLC analyses were performed on a Waters Acquity UPLC system using an Acquity UPLC column (BEH C18, 1.7 µm; 2.1 mm × 50 mm) a tuneable ultraviolet (TUV) detector at 254 nm, flow 0.2 ml.min^{-1}, injection volume 3 µl. Gradient elution: mobile phase A: 50 mM sodium acetate, pH = 6.6; mobile phase B: 0.2 M acetic acid/acetonitrile/methanol(1 : 4.5 : 4.5) (see Table 1 for gradient elution profile).

HPLC conditions

Liquid chromatography: Alliance 2695 with photodiode array 2996 detector and fluorescence 2475 detector (Waters, USA); chromatographic column: Zorbax Eclipse XBD C18, 150 × 4.6 mm, 5 µm (Agilent, USA); gradient elution mobile phase A: 0.2 M acetic acid/acetonitrile (9 : 1), mobile phase B: 0.2 M acetic acid/acetonitrile/methanol (1 : 4.5 : 4.5); flow 1 ml.min^{-1}; (see Table 1 for gradient elution profile); injection volume 10 µl; UV detection at 254 nm, fluorescence detection at λexc/λem = 330/500 nm.

Table 1. Gradient elution used in UPLC and HPLC methods

UPLC			HPLC		
Time (min)	**A (%)**	**B (%)**	**Time (min)**	**A (%)**	**B (%)**
0	50	50	**0**	40	60
4	20	60	**9**	30	70
7	0	100	**21**	0	100
8.40	5	95	**26**	0	100
10	50	50	**27**	40	60
			30	40	60

RESULTS AND DISCUSSION

BA in meat products were determined simultaneously by HPLC and UPLC (Table 2).

In comparison with HPLC, the UPLC system using small (sub-2 µm) particles in short columns (5 cm) significantly reduces analysis time without loss of efficiency. The time of analysis was reduced from 30 min in conventional HPLC to 10 min in UPLC. At the same, the economy and capacity of the system increased. The resolution of peaks was good and no interferences were observed. In the UPLC acetate sodium as a mobile phase proved to be better than acetic acid. The parameters of the presented UPLC method are comparable to those published earlier (1).

The content of BA in selected commodities (ham, the "Herkules" and "Poličan" sausage) ranged from units to tens

Table 2. Selected parameters-repeatability as RSD and retention time (Tr)

Biogenic amines	HPLC		UPLC	
	RSD (%)	**Tr (min)**	**RSD (%)**	**Tr (min)**
Spermine	6.99	25.2	0.91	7.9
Spermidine	5.60	22.0	0.89	6.8
Tyramine	3.52	20.6	0.80	6.4
Histamine	3.75	17.5	0.84	5.4
Cadaverine	5.67	16.3	0.79	5.2
Putrescine	6.15	15.4	0.93	5,0
2-phenylethyl-amine	5.44	13.9	0.77	4.8
Agmatine	3.56	12.0	1.27	4.4
Tryptamine	5.65	11.2	0.97	4.2

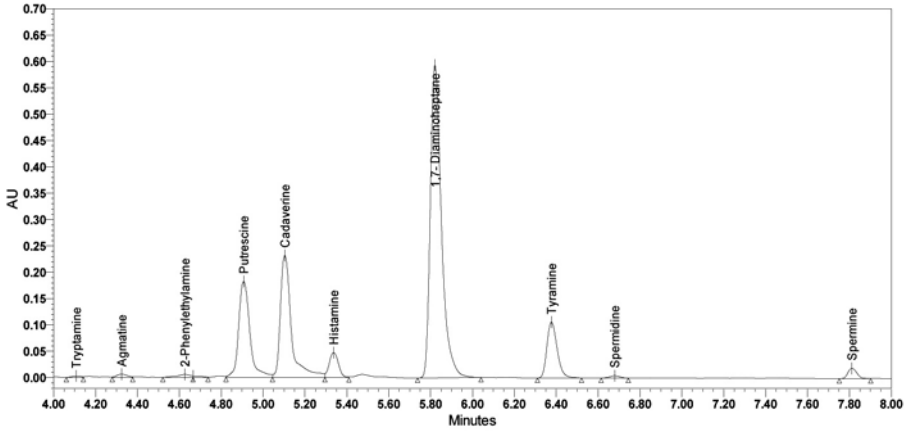

Fig. 1. Chromatogram of the fermented meat products

of mg.kg^{-1}. Values of the sum of BAs in cooked meat products were 2 to 3 times lower, namely 34 mg.kg^{-1} (08–62.7 mg.kg^{-1}), than in fermented products: 119.3 mg.kg^{-1} (3.2–275.6 mg.kg^{-1}). Most abundant were cadaverine (83 mg.kg^{-1}) and tyramine (48 mg.kg^{-1}) (Fig. 1). The results are in agreement with those reported by Karovičová and Kohajdová (2). The method is suitable for determination of nine BAs in foods of animal origin. As the BAs content in meat products of different type varied to a great extent, monitoring of BAs should be considered to ensure the quality and safety of meat products.

ACKNOWLEDGEMENTS

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FOLIA VETERINARIA, 55, Supplementum II: 47–49, 2011

THE ANTIOXIDANT INFLUENCE OF POWDERED AND LIQUID EXTRACT OF ROSEMARY ON FERMENTED MEAT PRODUCTS

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ABSTRACT

The prevention of an oxidation, yellowing and extension of a product longevity plays and always will play an important role in meat production. Rosemary extract is a very effective antioxidant. His principal activity is based on functional ingredients, such as rosemary acid, carnosic acid, carnosol, epirosmarol, betulinic acid, oleanolic acid, ursolic acid and triterpenic alcohols. The aim of the study was to monitor antioxidant influence of added rosemary extracts on "Malokarpatská" salami produced by Tauris company in cooperation with Trumf International and Kuk s.r.o. (DANISCO).

Key words: antioxidants; fermented meat products; rosemary extract

INTRODUCTION

The antioxidants are natural compounds which help to protect an organism against the harmful free radicals. Radicals are atoms or a group of atoms which can cause cell destruction or a weakening of the immune system which may lead to some diseases including many degenerative diseases, such as heart attacks or cancer. For these reasons antioxidants are important in prevention of diseases. Free radicals also induce an ageing process (1). The process of radical termination in some foodstuffs can produce substances which decreases their nutritional and sensorial value (aldehydes, ketones, etc.) (3). Many methods extending longevity of meat products, based on the use of antioxidants, have been used for a long time (the smoke used for curing contains antioxidants in a form of phenolic compounds) (6). The most active natural antioxidants are phenolic diterpenes, carnosic acid and carnosol, presenting approximately 15 % of commercial extracts of rosemary and more than 90 % of their antioxidant activity. Rosemary extract is a strong antioxidant which helps to remove the estrogens (prevention of breast cancer) (1). Fresh spice contain about 1–2 % of carnosic acid. The carnosic acid is unstable and converts to carnosol. Other active ingredients are rosmarol and rosmarinic acid (these belong to a large group of polyphenolic compounds) and epirosmarol (6). The antioxidative potential of vegetable materials (e.g. rosemary) is enhanced also by some triterpenic acids, such as betulinic acid, oleanolic acid, ursolic acid and triterpenic alcohols (7). Very remarkable property of rosemary is that its ingredient, the rosmarinic aci, is a stronger "interruptor" of superoxide radicals than ascorbic acid but, on the other hand, its activity in emulsions is lower (4).

MATERIALS AND METHODS

We monitored antioxidant influence of rosemary extracts added to the "Malokarpatská" salami, produced by Tauris company in cooperation with Trumf International and Kuk s.r.o. company (DANISCO). All the samples were compared with a standard "Malokarpatská" salami, made by Tauris Nitria spol. s r.o., Mojmírovce. We added rosemary as an antioxidant (affecting stability of fatty components) and a probiotic culture *Lactobacillus paracasei* into

the "Malokarpatská" salami. The scheme of a production was as follows:

Of all monitored parameters we present only peroxide number as it was the parameter most affected by addition of rosemary into the product. Fat was isolated from all samples by the Folch method (2) followed by determination of peroxide number (5). We evaluated 4 types of samples: standard "Malokarpatská" salami (Sample No. 1) as a control sample, Sample No. 2 (with powdered form of rosemary – TRUMF 1 g.kg^{-1}), Sample No. 3 (with liquid form of rosemary – KUK 0.4 g.kg^{-1}) and Sample No. 4 (with liquid form of rosemary – KUK 0.8 g.kg^{-1}).

RESULTS AND DISCUSSION

Oxidation progressed rapidly in Sample 1 and the peroxide number was increasing. This indicated onset of development of primary oxidation products. Samples 2 and 3 showed similar changes from day 21. Sample 2 exhibited narrower stability range. After day 14, the changes in Sample 3 resembled those in Sample 2 and starting from day 21, their curves of these samples were almost identical. The best results were achieved in Sample 4, that exhibited a wider range of stability. The dose of powdered rosemary extract (1 g.kg^{-1}) added to Sample 2 was the highest compared to other samples but still ensured only the same stability as that observed in Sample 3 (0.4 g of liquid extract of rosemary per 1 kg of the product). The last sample (No. 4) contained only 0.8 g of liquid rosemary extract per 1 kg of the product. The results obtained showed that the liquid form of the respective antioxidant ensured better stabilisation of the product and thus its increased longevity.

CONCLUSIONS

Fermented meat products usually contain more than 50 % of lipide components. As a result of this high content, we applied natural extracts of rosemary in powdered (Trumf s. r. o.) and liquid (Kuk s. r. o.) forms to samples investigated in our study. Samples No. 2, 3 and 4 were compared with the standard Sample No. 1 which served as a control. The best results were achieved with Sample 4 (0.8 g.kg^{-1} of rosemary), containing liquid rosemary extract (Kuk s. r. o.). The results confirmed positive effect of added antioxidant substances on stability of the product.

ACKNOWLEDGEMENTS

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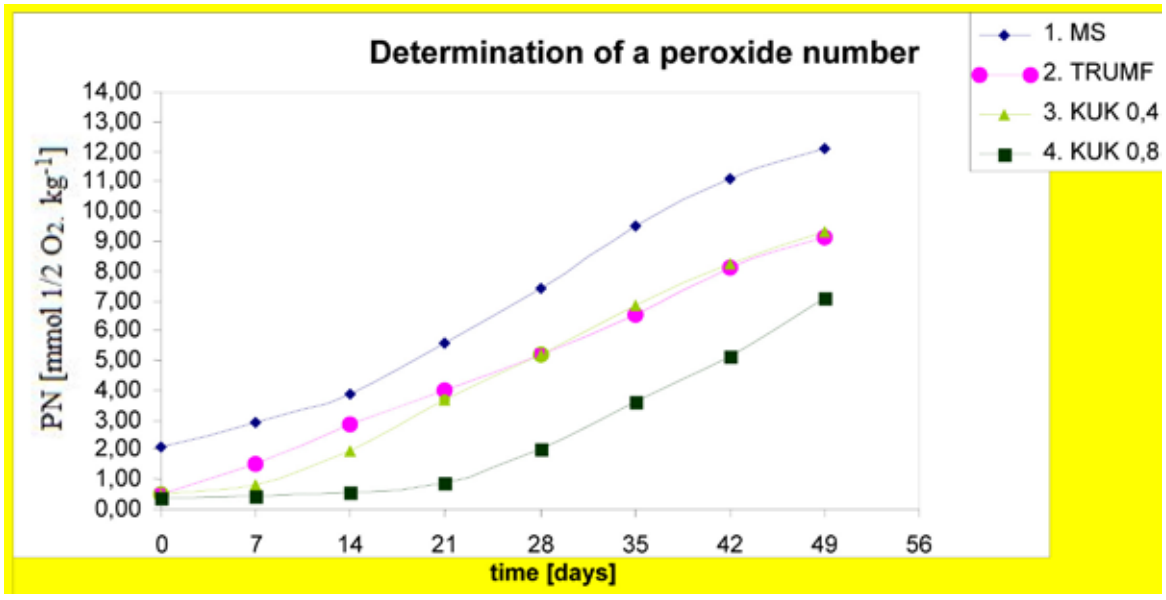

Fig. 1. Changes in peroxide number of samples in weekly intervals

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THE INFLUENCE OF SPECIFIC PROBIOTIC CULTURES ON PRODUCTION QUALITY OF FERMENTED MEAT PRODUCTS

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ABSTRACT

Probiotics containing foodstuffs are very popular nowadays even though a couple of the studies denied their health benefits. However, their technological benefits are undeniable. The most commonly used probiotic bacteria in a food production are *Bifidobacterium, Lactobacillus, Lactococcus* and some others. The aim of the study was to determine the influence of *Lactobacillus paracasei* on the stability of "Malokarpatská" salami, the survival of these probiotic microorganisms in this type of habitat and potential influence of rosemary extract on the presence of probiotic cultures.

Key words: fermented meat product; probiotics; rosemary; starter cultures

INTRODUCTION

Probiotics are defined as "Living microorganisms which when administered in adequate amounts confer a health benefit on the host" (3). Adequate amount is 10^6 colony forming units (CFU) per gram or ml of a product (2). This definition was presented by expert's FAO Committee (Food and Agriculture Organisation) in WHO (FAO/WHO) in 2002. Probiotic bacteria are used during the production of fermented salami. We recognize 3 phases of fermented salami production: formulation (creation of a product), fermentation and ageing. The rapid decrease in pH during fermentation is caused by lactic acid bacteria (*Lactobacillus plantarum, Pediococcus cerevisiae, Pediococcus acidilactici*). Lactic acid created by this process prevents the presence of other undesirable bacteria in the product what is, inter alia, important for development and stability of product colour. Fermentation could be achieved by spontaneously present bacteria or by using "starter cultures" (a special culture of microorganisms). Health requirements for a starter culture are rigorous and need to fulfil requirements for a GRAS substances (GRAS = Generally Recognised As Safe) (5).

The aim of this paper is to point to the importance of consumption of foods containing probiotic cultures (*Lactobacillus paracasei*).

MATERIALS AND METHODS

The goal of the study was to determine the influence of probiotic cultures of *Lactobacillus paracasei* on the stability of "Malokarpatská" salami and survival of probiotic microorganisms. Samples were taken from week 0, i. e. the time just after the allision of the raw meat into a casing. We monitored physico-chemical, microbiological and sensorial parameters in weekly intervals (6, 7).

Sample No. 1 – "Malokarpatská" salami, control sample (containing starter culture SAGA AF1® from Kerry Bio-Science USA);

Sample No. 2 – "Malokarpatská" salami with probiotic culture (*Lactobacillus paracasei* LYO 50 DCU from Kuk Slovakia spol. s r. o.);

Sample No. 3 – "Malokarpatská" salami with probiotic culture

(and with powdered rosemary extract from Trumf International s. r. o., at a dose of 1 g per 1 kg of a product);

Sample No. 4 – "Malokarpatská" salami with probiotic culture (and with liquid rosemary extract from KUK Slovakia s. r. o., at a dose of 0.4 g per 1 kg of a product);

Sample No. 5 – "Malokarpatská" salami with probiotic culture (and with liquid rosemary extract from KUK Slovakia s. r. o., at a dose of 0.8 g per 1 kg of a product).

RESULTS AND DISCUSSION

Results of our investigations are presented in Table 1.

CONCLUSIONS

Foodstuffs with a probiotic culture are currently very popular for their expected positive effect on gastrointestinal tract of the consumers (1). An important requirement for these products is a quantity presenting minimally 10^6 CFU of living probiotic microorganisms per gram of the product (4). We found 10^7 CFU of a culture of probiotic microflora per gram of the product from day 7 up to the end of the experiment which is a really positive finding. Addition of the rosemary extract (powdered or liquid) had no effect on the presence of probiotic culture. Other monitored parameters in all the samples complied with Codex Alimentarius of the Slovak Republic.

Table 1. Results of microbiological examination of salami samples

		MRS	VRBL	SLM	LMO	*Listeria* sp.
1. Sample	day 0	1.9×10^5	0	neg	neg	neg
	day 7	6.7×10^7	0	–	–	
	day 21	7.8×10^7	0	neg	neg	
	day 28	8.5×10^7	0	neg	neg	
		MRS	**VRBL**	**SLM**	**LMO**	***Listeria* sp.**
2. Sample	day 0	1.1×10^6	10	neg	neg	neg
	day 7	2.9×10^7	0	–	–	
	day 21	3.7×10^7	0	neg	neg	
	day 28	3.5×10^7	0	neg	neg	
		MRS	**VRBL**	**SLM**	**LMO**	***Listeria* sp.**
3. Sample	day 0	9.7×10^5	10	neg	neg	neg
	day 7	4.6×10^7	0	–	–	
	day 21	5.2×10^7	0	neg	neg	
	day 28	2.9×10^7	0	neg	neg	
		MRS	**VRBL**	**SLM**	**LMO**	***Listeria* sp.**
4. Sample	day 0	9.8×10^5	0	neg	neg	*L. welshimeri*
	day 7	5.5×10^7	0	–	–	
	day 21	4.2×10^7	0	neg	neg	
	day 28	1.3×10^7	0	neg	neg	
		MRS	**VRBL**	**SLM**	**LMO**	***Listeria* sp.**
5. Sample	day 0	8.3×10^5	50	neg	neg	neg
	day 7	7.1×10^7	0	–	–	
	day 21	4.6×10^7	0	neg	neg	
	day 28	1.4×10^7	0	neg	neg	

MRS – agar for *Lactobacillus*; VRBL– agar for coliform bacteria
SLM – presence of *Salmonella* sp.; LMO – presence of *Listeria monocytogenes*

ACKNOWLEDGEMENTS

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THE QUALITY OF ANIMAL MEAT IN COMPARISON WITH SOY MEAT SUBSTITUTE REGARDING THE CONTENT OF ESSENTIAL ELEMENTS MANGANESE, COPPER, IRON AND ZINC

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ABSTRACT

We compared the quality of pork and beef meat with soy meat substitutes in terms of content of essential elements manganese, copper, iron and zinc. Soy meat from the considered point of view is an equivalent substitution for pork and beef meat regarding the fact that it contains more manganese and less zinc.

Key words: beef; copper; ICP; iron; manganese; pork, soya meat; zinc

INTRODUCTION

Beef and pork play an important role in the diet of people because they are source of animal protein and unalterable nutritional substances necessary for the proper functioning of the human organism. Many people are replacing quality animal meat with vegetable ersatz meat for various reasons. The food market offers soya meat in the form of soya cubes, soya slices and soya flakes. Essentially, it is textured defatted soya flour containing about 50 % proteins, 31 % carbohydrates, 17 % fibre and 2 % fat, as stated by the manufacturer's label. The starting raw material for the manufacturing of the mentioned ersatz meat is soya beans (*Glycine max L.*). It is legume which contains on average 40 % proteins of which 6 % are anti-nutrients known as inhibitors of proteinase (1, 2). The proteinase inhibitors are partially inactivated by heat treatment, which improves the quality of these proteins.

The aim of this study was to compare the quality of pork and beef with soya meat substitute in terms of content of essential elements manganese, copper, iron and zinc.

MATERIAL AND METHODS

Essential elements manganese, copper, iron, and zinc were determined in samples of pork and beef and of soya meat (soya cubes, soya flakes). The material was purchased at a grocery store. Prior to the analysis, the sample was mineralised in a microwave apparatus MW FLYWHEEL-2 BERGHOF (Germany). The weight of the samples ranged between 0.3 and 1.0 g of the material, the resulting volume was 25 ml. Conventional analysis was carried out by accredited methods at the State Veterinary and Food Administration of the Slovak Republic in Košice, employing apparatus AGILENT 7500c ICP-MS (AGILENT, USA).

RESULTS AND DISCUSSION

Results of the analyses are presented in Tables 1 and 2 and in Figures 1A, B, C, D.

In this study we analysed 29 samples of pork and 37 samples of beef meat. The content of essential elements in pork and beef meat fluctuated to a large extent as witnessed by values given in Table 1.

The average content of Mn, Cu, Fe and Zn measured in

Table 1. The content of manganese, copper, iron and zinc in pork and beef meat

Commodity		Concentration of essential elements in meat (mg.kg^{-1})			
		Mn	**Cu**	**Fe**	**Zn**
Pork muscle	Min	0.074	0.305	5.339	8.383
	Max	0.693	2.288	56.02	60.82
	Mean	**0.306**	**1.286**	**16.17**	**27.19**
	SD	0.209	0.644	10.35	13.75
	n	11	29	29	29
Beef muscle	Min	0.022	0.302	8.131	21.82
	Max	0.658	1.991	50.97	73.85
	Mean	**0.146**	**0.995**	**25.98**	**48.59**
	SD	0.153	0.477	9.028	13.55
	n	17	37	37	37

SD – standard deviation; n – number of samples

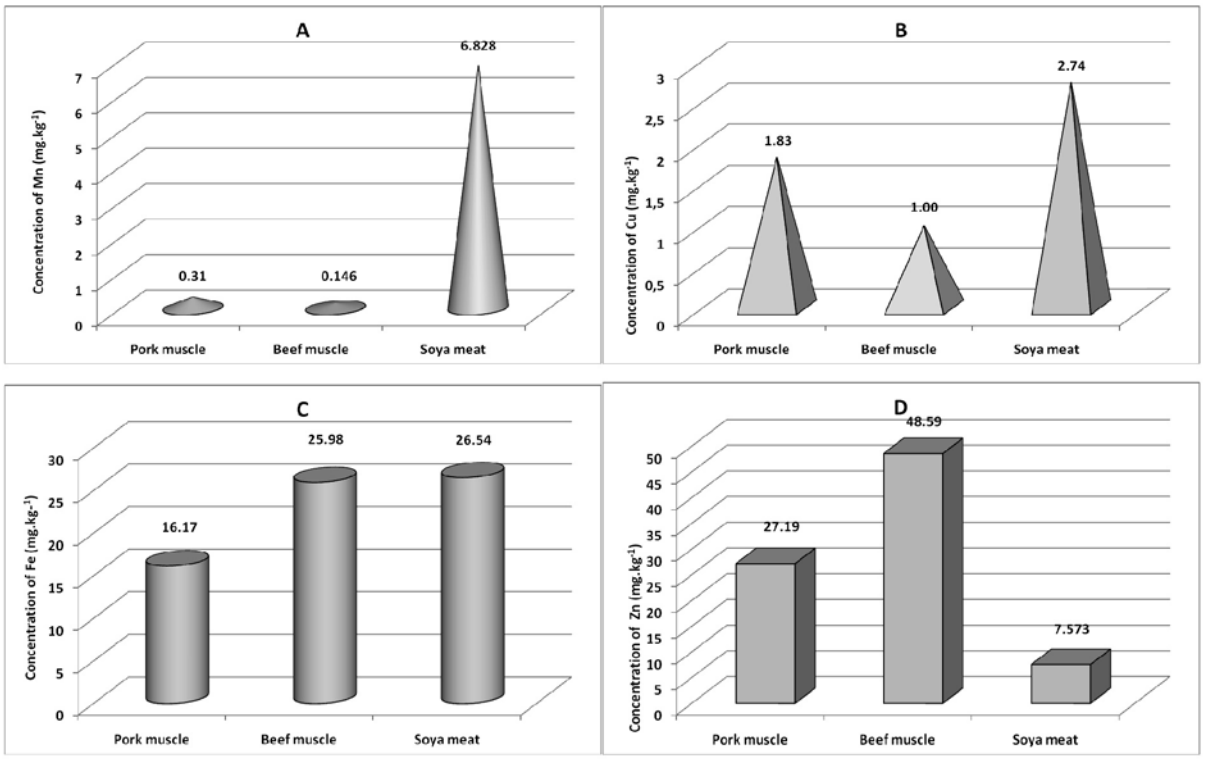

Fig. 1. A comparison of the average concentration of Mn (Fig. 1A), Cu (Fig. 1B), Fe (Fig. 1C) and Zn (Fig. 1D) in the animal meat and an average concentration of these elements in plant (soya) meat substitute

Table 2. Comparison of the average content of manganese, copper, iron and zinc in pork, beef and soya meat substitute

Commodity	Mn ($mg.kg^{-1}$)	Cu ($mg.kg^{-1}$)	Fe ($mg.kg^{-1}$)	Zn ($mg.kg^{-1}$)
Pork muscle	0.306	1.286	16.17	27.19
Beef muscle	0.146	0.995	25.98	48.59
Soya slices	6.556	2.81	28.88	7.805
Soya cubes	7.099	2.662	24.2	7.341

the selected samples of pork meat differed from the average content of these elements in the selected samples of beef meat. The average content of Mn, Cu and Zn in the samples of soya cubes and soya slices was similar. The difference of about 5 mg was recorded only for the content of Fe in these samples (Table 2).

The Figure 1A shows that soya meat supplement contained on average 47-fold more manganese than beef and nearly 22-fold more than pork; pork contained 2-fold more Mn than beef, which probably reflects the higher intake of Mn from mineral compound feedstuffs by swine compared to the cattle. Velíšek (3) reported that the content of Mn in pork was in the range 0.12–0.18 $mg.kg^{-1}$, which is lower than the average value of 0.306 $mg.kg^{-1}$ referred to in this paper. For beef, our results are comparable with the published results. The content of Mn in soya beans has been stated to be in the range of 14–90 $mg.kg^{-1}$ (3). This value is much higher than that presented in our study for soya meat, which is however realistic, because soya meat supplement is a product from soya beans. The Figure 1B shows that soya meat supplement contained on average 2.75-fold more copper than beef and 1.5-fold more than the pork; pork contained approximately 1.8-fold more Cu than beef. The content of iron in soya meat supplement resembles that of beef. Pork contained 1.6-fold less Fe than beef and soya meat supplement (Fig. 1C). Soya meat supplement showed 6.4–3.6-times less zinc content in comparison with the bovine and porcine meat. Beef shows a 1.8-fold higher content of Zn than pork (Fig. 1D). The results for the content of Cu, Fe and Zn in pork and beef published in this study are in good compliance with the values published in the literature (3) except for the content of Zn in beef, where the average value of 48.59 $mg.kg^{-1}$ is higher than the level reported by the respective author (30–43 $mg\ kg^{-1}$).

CONCLUSIONS

We observed high variability of content of the investigated elements in individual samples of pork and beef. In term of the content of the essential elements manganese, copper, zinc and iron, soya meat substitute may, under certain conditions, fully replace the beef or pork.

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DETECTION OF MEAT FREEZING

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ABSTRACT

The detection of meat freezing is important from two points of view, firstly the possible adulteration of meat and meat products and secondly the control if the meat was frozen before it was used for preparation of raw meat products (tea sausage, steak tartar). Different methods can be used; some of them are based on the measurement of the activity of mitochondrial enzymes. The enzyme activity of citrate synthase and aconitase was tested with commercial sets to prove whether various samples (pork, beef, chicken) stored under different storage conditions were frozen or not. The freezing of the meat caused a considerable increase in the activity of both enzymes. A certain small increase in the enzyme activity was observed at the fresh meat samples, which were stored for a longer period at 4 °C. This could be caused by microbial spoilage.

Key words: adulteration of meat; aconitase; citrate synthase; meat freezing; mitochondrial enzymes

INTRODUCTION

To prove freezing of meat or to monitor its extent shows to be important mainly for possible adulteration or the necessity to verify whether raw material (meat) used for non heated products was really frozen to eliminate potential parasites (trichines, toxoplasma, etc.) (7). For this purpose, many methods (chemical or physical) have been developed. They have been used mainly for fish, where the freezing procedure is most likely applied and has a huge significance in detecting frozen fish on the fresh fish market. A variety of methods using analysis of meat exudate based on activity measurement of some enzymes has been developed. Other methods include isoelectric focusation, reflective spectrophotometry in infrared area, cryoscopy or magnetic resonance. However, it seems that the detection of freezing by enzymes is predominant.

Enzyme methods evaluate the activity of enzymes that are not present in the meat exudate under normal circumstances unlike in thawed meat. In most cases enzymes are normally present in cell organelles; the destruction of these organelles caused by ice crystals results in the enzyme release and it is therefore possible to detect their activity in the meat exudate.

Such enzymes are especially the enzymes of the citric acid cycle. They are found particularly in the mitochondria, from which they are released after the outer membrane damage and occur then in the meat juice. Many techniques detecting enzyme activity (citrate synthase, aconitase, ATPsynthase, fumarase, lipoamide dehydrogenase and 3-β-Hydroxyacyl-CoA-dehydrogenase (HADH)) (4) proving meat freezing procedures have been developed. This type of mitochondrial enzyme detection of frozen/thawed meat is commonly used for fish freshness control. Although foreign studies present the application of these methods even for pork (1, 2), these specific methods are not used for meat of big mammals in the Czech Republic. However, it shows that relevant enzyme assay kits are applicable for pork and even for beef (5). The aim of the study was to verify if it is possible to distinguish fresh from thawed meat by the change of the catalytic activity of citrate synthase (CS) and aconitase (AC) and to evaluate which factors can affect the process.

In the work itself we studied if the commercial sets for the activity measurement of mentioned enzymes are possible to use, and whether these methods work for common types of meat, such as beef. Following results are the summary of individual experiments.

MATERIALS AND METHODS

Meat samples were obtained from beef *longissimus lumborum et thoracis* muscle, which was sliced, packed (LD-PE sealed plastic bag) and stored under two different storage conditions; refrigeration temperature 4 °C and freezing temperature –18 °C. For each measurement two identical samples were used. The activity of both mentioned enzymes was determined in exudate from fresh, refrigerated and frozen/thawed meat.

The activity of citrate synthase was measured by a spectrophotometric method. After the release of acetyl coenzyme A and addition 5,5´-dithiobis-(2-nitrobenzoic) acid (DTNB), CoA-S-S-TNB and yellow 5-thio-2-nitrobenzoic acid (TNB) are formed. The concentration (absorbance at 412 nm) of TNB corresponds with the activity of CS. Firstly, the endogenous activity after 20 s of lag time is obtained and then the actual measurement followed with duration 90 s in 10 s intervals. For the experiment, we used a commercial assay kit Sigma Aldrich (Germany).

The activity of aconitase was also measured spectrophometrically. AC catalyses the reversible isomerization of citrate to isocitrate via the intermediate, *cis*-aconitate. The activity of AC was evaluated at 340 nm by measuring the change of absorbance caused by the conversion of NADP to NADPH + H^+ at 37 °C (8).

RESULTS

The data confirmed that the freezing of meat significantly increases the activity of CS and AC. The activity of both enzymes was influenced by the freezing process approximately in the same way. In other experiment (6), repeated freeze-thaw cycle resulted in the activity increase, every cycle led to further increase.

Refrigeration storage of fresh meat at 4 °C did not significantly influence the activities; however, after a longer storage period, microbial deterioration occurred and consequently increased the activity of CS. The meat structure may be damaged by present microorganisms, which leads to enzyme release from muscle organelles. The presence of bacterial CS might show some activity on its own and therefore may affect the results. Nevertheless, microbial destruction of meat tissue indicates meat decay, and it is no longer necessary to prove whether the meat was frozen (5).

It was also compared how the activity of CS from meat exudate varies, when the outer layers of meat are partially frozen after passing through the freezing tunnel (Froster). With the increasing duration of time in the tunnel the frozen layer was logically deeper and the tissue damage and the activity of enzyme increased. After packaging and storage at 4 °C, the temperature between the unfrozen core and the frozen surface and its surroundings was equalized. As the consequence, the activity of CS during further refrigerated storage also changed (6).

Interesting is the comparision of the CS (Fig. 1) and AC (Fig. 2) activity during refrigerated and frozen storage of beef. Whereas the activity of CS did not significantly vary, the difference between the activity of AC of refrigerated and frozen meat was higher.

CONCLUSION

The paper showed practical applicability of the method for detection of fresh and frozen/thawed beef meat based on measuring the activity of citrate synthase and aconitase. During long term refrigerated storage, the meat products undergo decay or microbial contamination can result in higher enzyme activity. As a result, cell organelles are damaged by their own enzymes instead of ice crystals and interfere with the detection. In case the meat has already been decayed, the detection of possible previous freezing is out of the question.

ACKNOWLEDGEMENT

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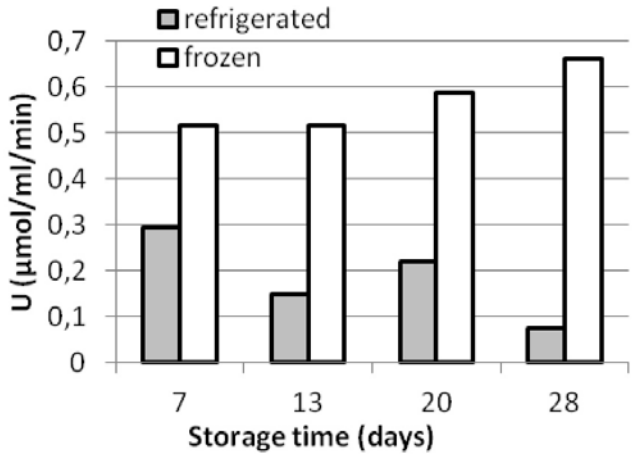

Fig. 1. Catalytic activity of citrate synthase during refrigerated and frozen storage of beef

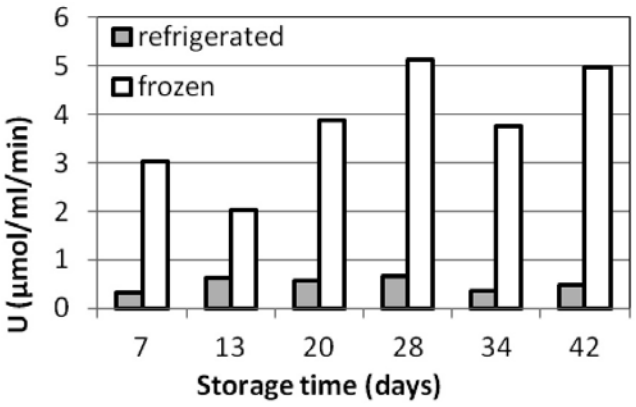

Fig. 2. Catalytic activity of aconitase during refrigerated and frozen storage of beef

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COMPARISON OF NUTRITIONAL QUALITY OF MEAT OF CALVES FROM MILK FATTENING AND BABY-BEEF FATTENING

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ABSTRACT

We compared 2 types of fattening of Holstein bulls. In the milk fattening (during 13 weeks) the first group received *ad libitum* MKZ 1 (containing 15% sunflower oil – more unsaturated fatty acids) and the second group MKZ 2 (containing 15% beef tallow – less unsaturated fatty acids), lucerne hay in minimum doses of 100–200 g/head per day. Fattening baby-beef received *ad libitum* water, granular feed mixture and lucerne hay in amounts up to 25% of the total ration. The higher total fat content (MKZ 1 – 1.02 and MKZ 2 –1.3 g.100g^{-1}) indicated higher energy value of MKZ 2 meat (+3.65%, P < 0.001). We confirmed that extending the fattening increases the fat content of meat in bulls from baby-beef fattening (1.567 g.100g^{-1}) and the energy value (448.015 kJ.100g^{-1}). We confirmed the effect of feeding milk replacers with different content of fatty acids on the composition of fatty acids in intramuscular fat of *musculus longissimus thoracis et lumborum*.

Key words: calves; fattening baby-beef; fatty acids; milk fattening

INTRODUCTION

Compared with the neighbouring states, the total meat consumption in Slovakia, has been lowest since 1990 and has a downward trend to the current 58.6 kg per person (a decrease of 30.2%). Out of this is the annual beef consumption is only 4.4 kg per person and is falling every year. In Slovakia, beef rearing is focused on milk production. Bulls of dairy breeds are less suitable for fattening to the classical higher carcass slaughter weight because of worse carcass data, such as fat and kidney fat (4). The starting point may be milk fattening or baby beef fattening. For producers and processors the carcass quality is a particularly important issue, i. e. the proportion of muscle, fat and bone tissues. For the consumer, however, essential are the sensory properties of meat and nutritional physiological criteria, the hygienic and toxicological aspects – content of residues (5). Mojto and Zaujec (7) referred fat to be the most variable component of bovine meat. Fat from meat contains a high proportion of saturated fatty acids. Important source of saturated fatty acids are also milk fat and hydrogenated vegetable fats (11). Composition of fat in muscles of fattening calves showed that feeding different rations causes different composition of fat in the muscles (1).

The aim of this paper was to evaluate and compare the nutritional quality of meat from calves fed milk replacer with fat from various sources and baby-beef fattening.

MATERIAL AND METHODS

Milk fattening of Holstein calves (n = 21) was done on the Experimental Farm at APRC Nitra. All calves were fed according to the same feeding schedule for 13 weeks of fattening, The first experimental group received milk replacer MKZ 1 (containing 15% sunflower oil with more unsaturated fatty acids) and the second experimental group was fed MKZ 2 (containing 15% beef tallow with less unsaturated fatty acids). From the second week of the trial, both milk replacers were fed *ad libitum* and lucerne hay in minimal doses 100–200 g/head per day. The main parts of the milk replacer were skimmed milk powder (58.5%) and fat (20%). Information on the content of nutrients and composition of milk replacers can be obtained from the authors. Calves were fed milk replacers twice daily.

Baby-beef fattening was carried out on the farm VKK Veľké Hoste, company MVL Agro Ltd., Malé Chlievany, from September to February. After the milk nutrition period, the Holstein calves (n = 27) were housed outdoors in pens with straw bedding in groups of 4 to 5, and later on in groups of 3. In winter they were housed together in one pen. The animals were provided *libitum* granulated feed mixture and water and lucerne hay in quantities up to 25 % of the total ration. Information on the content of nutrients and composition of granulated mixed feed can be obtained from the authors. After the fattening, we selected six steers with balanced LW for slaughter and analysis of carcass data at the experimental slaughterhouse at APRC Nitra, in accordance with the standard STN 46 6120.

All calves were analyzed for a range of indicators of nutritional meat quality and intramuscular fat (*m. longissimus thoracis et lumborum*). We determined the content of water (a microwave analyzer ULTRA-X), fat, ash and proteins according to the STN 57 0185, and the content of cholesterol by photometric method of Horňáková *et al.* (3). The content of fatty acids in intramuscular fat was determined by a gas chromatograph Carlo Erba GC 8000 Top. Then we calculated two indices: the index of unsaturated fat (I_1) as a ratio of unsaturated and saturated fatty acids (Σ MK $_{1,2,3}$/Σ MK_O) and the index of nutritional value of fat (I_2) as a ratio of essential and saturated fatty acid percentages of individual fatty acids (å $MK_{1,2}$/å MK_O) according to Zembayashi *et al.* (12). We used a professional statistical software SAS, version 9.2 (9), module SAS STAT, to evaluate the results obtained during milk fattening. The results obtained by baby-beef fattening were used to calculate the statistical characteristics: the mean, standard deviation and coefficient of variation.

RESULTS AND DISCUSSION

At the end of the milk fattening, the MKZ 1 animals reached 126.36 kg at the age of 91 days and MKZ 2 animals 152.30 kg. Bulls from baby-beef fattening reached the average live weight of 278.41 kg at the age of 245 days. From these parameters of nutritional meat quality (Table 1) we found out that the MKZ 2 group (milk replacer with higher content of saturated fatty acids) showed significantly higher fat content in meat and consequently also energy value. In bulls from baby-beef fattening we observed higher proportion of protein, fat and a higher energy value of meat when compared with milk fattening. We agree with Mojto *et al.* (6) that with increasing age the values of fat increase at the expense of water content. When we compared the results of milk fattening with the results of Skřivanová *et al.* (10), we observed minimum differences in the protein content (2.8 %) but higher levels of fat content, by 28 % in group MKZ 1, and by 43.85 % in group MKZ 2 at a comparable age of animals. However, the final LW reported by the above mentioned authors in calves (141.4 kg) was higher than in the group MKZ 1 and lower in MKZ 2. We found higher proportion of fat (by 2–3 %) in comparison with Nosáľ *et al.* (8). Comparable results of fat content and energy value with the group MKZ 1 were observed by Mojto *et al.* (7). Čuboň *et al.* (2) detected lower protein and higher fat content and thus also higher energy value than we observed in bulls from baby-beef fattening.

We found significant differences between groups (59.34 mg.100 g^{-1} with MKZ 1 and 69.10 mg.100 g^{-1} with MKZ 2) in milk fattening. We obtained higher values compared with the results of Skřivanová *et al.* (10). The MKZ 1 group reached lower values and MKZ 2 similar values as Mojto *et al.* (7). The intramuscular fat of *m. longissimus thoracis et lumborum* contained the highest proportion of oleic acid and linoleic acid in both groups of milk fattening. In baby-beef fattening, the monounsaturated fatty oleic acid content was the highest out of all observed fatty acids, its content was comparable with that in the group MKZ 2. The MKZ 1 group contained significantly more unsaturated essential fatty acids in the meat (linoleic acid, arachidonic acid). The group MKZ 2 had significantly more monounsaturated fatty

Table 1. Comparison of the chemical composition of the basic indicators of nutritional quality in meat from milk fattening

Group		MKZ 1 n = 11		MKZ 2 n = 10		
Indicator	**Unit**	$\bar{x}$	s_x	$\bar{x}$	s_x	**T-test**
Total water content	g.100g $^{-1}$	77.09	0.22	76.74	0.28	-
Total protein content	g.100g $^{-1}$	20.97	0.31	21.22	0.39	-
Total fat content	g.100g $^{-1}$	1.02	0.08	1.30	0.22	+++
Ash content	g.100g $^{-1}$	1.02	0.04	1.02	0.04	-
Energy value	kJ.100g^{-1}	389.65	6.92	404.42	9.90	+++
Cholesterol content	mg.100g^{-1}	59.34	7.31	69.10	7.10	++

+ – $P < 0.05$; ++ – $P < 0.01$; +++ – $P < 0.001$

acids (palmitoleic, oleic acid) and saturated fatty acids $C_{14:0}$ and $C_{18:0}$. The proportion of palmitic and stearic acids was the highest out of all saturated fatty acids. Similar trends were also found in baby-beef fattening, where the content of palmitic acid was higher by one third in line with Mojto *et al.* (7). The proportion of linoleic acid was the highest of all essential fatty acids. Linolenic acid and arachidonic acid were present in minimal amounts in meat, which corresponded to the results of other authors (6). The baby-beef bulls also had the highest content of linoleic acid out of essential fatty acid, but compared with group MKZ 1 it was 4.3-fold lower, and compared with MKZ it was 2–2.4-fold lower. We confirmed the effect of feeding different sources of fat in the milk replacer on resulting ratio of saturated and unsaturated fatty acids in meat of investigated animals. The calves in the group MKZ 1 reached significantly higher ($P < 0.001$) index of unsaturated fat (MKZ 1 to 2.54; MKZ 2 to 2.08) as well as the nutritional value of fat (MKZ1 – 1.35; MKZ 2 to 0.67).

Our levels of unsaturated fatty acids were much higher than those reported by other authors (6, 7, 10). Comparable values in Holstein steers were reached by Mojto *et al.* (7) with respect to the index unsaturated fat only. Our results of the representation of individual fatty acids in baby-beef fattening also showed low index of unsaturated fat (1.32) and index of the nutritional value of fat (0.21).

CONCLUSION

Currently the milk fattening of calves is economically inefficient (expensive dairy ingredients) in Slovakia. However, it offers nutritionally valuable meat that the consumer would certainly appreciate. We found out that baby-beef fattening is a good alternative to the use of bulls of dairy breeds with very good carcass data, but the composition of fatty acids in intramuscular fat does not fulfil the standards of dietary recommendations, namely the ratio of polyunsaturated to saturated fatty acids to be at least 0.4.

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EFFICACY OF ACTION OF CAPRYLIC ACID AND HYDROGEN PEROXIDE ON SELECTED TYPES OF MICROMYCETS

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ABSTRACT

The aim of this study, focused on development of a combined broad-spectrum disinfectant, was to investigate antifungal efficacy of two chemical agents in varying concentrations, namely caprylic acid and hydrogen peroxide (6%, 5%, 3%, 2%, 1%, 0.5%, 0.3%, 0.1%). The disinfectant efficacy was tested on collection strains of microscopical fibrous fungi *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus* and *Penicillium* spp. The fungicidal activity was tested by the method STN EN 1650. The method used was supplemented with disc diffusion method. Not all selected concentration showed 100 % effectiveness against selected micromycetes.

Key words: caprylic acid; disinfection; hydrogen peroxide; micromycets

INTRODUCTION

Presently increased attention is paid to food safety and the related indicators of risk factors in components entering the food chain. One of such risk factors is the occurrence of potentially toxinogenic species of microscopic fungi that are bound by their life cycle to these substrates (8). Due to their morphological and physiological variability and flexibility, micromycetes may occur in various ecological systems in the soil, water, air and foodstuffs. Micromycetes are involved in modern technologies of production of medicines, organic acids, enzymes, fatty acids, amino acids, vitamins and similar (4). They may be pathogenic to plants and induce mycotic diseases in animals and humans, the so-called mycoses (2, 11). It was confirmed that some micromycetes occurring in food (e.g. Mucor) may cause alimentary diseases (3). Their presence should be controlled so as their beneficial action is not negated by their adverse effects. Thus disinfection and disinfectants became important tools in protection of human health and in all food industry operations (7). The most effective chemical disinfectants include those with strong oxidative effects. They act on almost the whole range of important pathogens not excluding the spores (10). Acids are effective disinfectants, however, due to their adverse properties (corrosion, irritation) their sole use is limited (7).

The aim of our still continuing study is to develop a combined chemical disinfectant with bactericidal and fungicidal effect. The first stage in this effort was determination of minimum fungicidal efficacy which was the subject of the present experiments.

MATERIALS AND METHODS

We tested the following chemical agents: caprylic acid and hydrogen peroxide in concentrations 6%, 5%, 3%, 2%, 1%, 0.5%, 0.3%, 0.1%. Disinfectant efficacy of the two chemicals was tested with the following strains of fibrous fungi: *Aspergillus flavus* (CCM 8363), *Aspergillus parasiticus* (CCM F-108), *Aspergillus ochraceus* (CCM F-269), *Penicillium* spp. (CCM 8259), obtained from the Czech Collection of Micro-organisms (Masaryk University, Brno, CR). Antifungal activity of caprylic acid and hydrogen peroxide was tested by the method STN EN 1650 (9) and disc diffusion method (1).

RESULTS AND DISCUSSION

Results obtained in this study by means of suspension and disc diffusion tests are presented in Table 1 and Table 2.

Table 1. Efficacy of caprylic acid and hydrogen peroxide against micromycetes – suspension method

	Micromycetes (CFU.ml^{-1})							
	Asp. flavus		*Asp. Parasiticus*		*Asp. ochraceus*		*Penicillium* spp.	
CFU.ml^{-1*}	1.21 x 10^7		1.36 x 10^7		9.6 x 10^7		1.01 x 10^7	
Conc. CHS (%)	HP	CA	HP	CA	HP	CA	HP	CA
6	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
2	0	0	0	0	0	1.0×10^5	1.0×10^5	0
1	1.0×10^5	1.0×10^5	1.5×10^5	3.0×10^5	2.0×10^5	1.0×10^5	4.0×10^5	2.0×10^5
0.5	4.6×10^6	1.0×10^5	2.0×10^5	2.0×10^5	2.0×10^5	6.0×10^5	6.0×10^5	3.0×10^5

CHA – chemical substances; * – colony count before use of CHS; CFU – colony forming units; HP – hydrogen peroxide; CA – caprylic acid;

Table 2. Efficacy of caprylic acid and hydrogen peroxide against micromycetes – disc diffusion method

	Micromycetes (inhibition zones in mm)							
	Asp. flavus		*Asp. Parasiticus*		*Asp. Ochraceus*		*Penicillium* spp.	
Conc. CHS (%)	HP	CA	HP	CA	HP	CA	HP	CA
6	36	40	38	40	36	40	34	36
5	36	38	36	38	30	38	30	36
3	24	40	30	38	28	38	26	26
2	20	24	24	26	26	20	20	20
1	20	20	20	17	17	20	19	16
0.5	17	20	19	19	16	17	16	16

CHA – chemical substances; HP – hydrogen peroxide
CA – caprylic acid; substance/concentration producing inhibition zone >20 mm was considered effective

After 15 min exposure of micromycetes to chemical substances, we observed 100 % efficacy of both agents at concentrations of 6 %, 5 % and 3 % (Tab. 1 and 2) while 2 % concentration failed to affect *Penicillium* spp. and *Aspergillus ochraceus*, as determined by both methods. None of the tested strains was affected by concentrations 1 % and 0.5 % as none of the inhibition zones was greater than 20 mm at these concentrations.

It is known that fatty acids have rather good antimicrobial action against broad spectrum of pathogens including bacteria, viruses and parasites. Caprylic acid (octanoic acid) is fatty acid with eight carbon atoms and occurs naturally in mother's milk, cow milk and also in coconut oil. It is generally considered safe for use in food processing (6).

Hydrogen peroxide is a very strong oxidant effective against broad spectrum of micro-organisms including bacteria yeasts, moulds and viruses. The target effective concentration of hydrogen peroxide ranges between 50 and 100 ppm (5). In our study, the target concentration against micromycetes was approximately 2 %.

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