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INFLUENCE OF DIFFERENT CURING METHODS ON THE FATTY ACID COMPOSITION IN SAUSAGES PREPARED FROM RED DEER MEAT

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ABSTRACT

These curing agents play a decisive role in obtaining the specific sensory properties, stability and hygienic safety of products such as fermented sausages, ham and, more recently, emulsion type of sausages. The effect of using two different curing agents (sodium chloride and nitrate) on fatty acid compounds in dry-cured deer meat was investigated in our study. The concentration of free fatty acids in the fat depends on the hydrolytic activity of the lipases, the microbial metabolic processes, and the oxidative reactions that work on the free fatty acids released in the lipolysis. The main identified fatty acids in all different types of curing were palmitic acid (16 : 0), oleic acid (c18 : 1 cis-9), stearic acid (C18 : 0). The resulting *n*-6/*n*-3 PUFA ratio in the muscle samples of red deer showed no variation in different types of curing and was beneficially low within the range of 3.9 : 1 and 4.49 : 1. Total free fatty acids, whether saturated, monounsaturated or polyunsaturated fatty acids, did not increased (p >0.05) greatly through the processing of dry-cured deer meat. Also there was no effect of curing method on fatty acids composition in two different muscles *Semitendinosus muscle* (ANOVA, p >0.05, F – 0.003, F crit. – 3.041) and *Triceps brachii* muscle (ANOVA, p >0.05, F – 0.05, F crit. – 3.01). There were found no significant (p >0.05) differences between fatty acids content in sausages prepared by brining in NaCl and Nitrate salt. The present study revealed that game meat can function as a good source of bioactive compounds that are essential for human nutrition.

Keywords: red deer; fatty acids composition; curing

INTRODUCTION

Concentration of fatty acids determines not only nutritional value of the meat, but also its quality, including its shelf life and taste **(Wood et al., 2003; Warren et al., 2008)**. Game meat has characteristic texture and taste: it is generally darker, has a stronger taste and is often tougher than meat from farm animals. Although game meat is considered more difficult to prepare, when purchased raw, current trend towards processed products, such as fermented sausages, is a new aspect to take into account.

Deer (*Cervidae*) belong to the most important species, which are used as a farm animal as well as hunting wild animal. For long-term conservation and development purposes, it therefore appears compulsory to manage wildlife to maintain both species survival and within species genetic diversity **(Franklin, 1980; Maršálková et al., 2014)**.

Deer farming meets the growing interest which consumers show, for meat alternatives to the traditional types **(Volpelli et al., 2003)**: venison is appreciated because it is lean and tasty but, even more, as stressed by **Barry (1994)**, because it fulfils the needs of "a society which is becoming increasingly sensitive to environmental pollution, animal manipulation, and feed additives" **(Belej et al., 2011)**.

In the last 10 years, cured, fermented and dried products made from hunted game species have become more popular and are easily found on the market in European countries. Game meat is distinguished by its characteristic texture and taste: it is generally darker, stronger-tasting and often tougher than that of poultry and farmyard animals. **(Bertolini et al., 2005; Hoffman and Wiklund, 2006; Ruiz et al., 2007)**. Cured, fermented and dried products made from game species have recently appeared on the market. However, their presence remains very restricted, and is highly seasonal. Marketing of venison, for example, is dependent on the timing of the hunting season and on culling quotas. In addition, the quality of game products depends upon the raw meat quality, which is influenced by, among others factors, the period of the year in which the animals are growing, that affects the condition of the pasture and the animal's activity and sexual activity. As an example, the fat reserves of venison are decreased after the mating period in autumn **(Ruiz et al., 2007)**.

In the human diet, meat is seen as a major source of fat, and especially of saturated fatty acids (SFAs), which have been implicated in diseases associated with modern life (various cancers and coronary heart disease), mostly in developed countries. The World Health Organization recommends that the daily fat intake be reduced to 30% of the total energy intake, and that saturated fats should be limited to 10% of this caloric intake **(Krauss et al., 2000)**.

Curing is one of the most ancient meat preservation methods, which contributes positively to the technological and sensory characteristics of the meat. The curing technology is based on the addition of salt, which acts as a preserving agent and is also responsible for causing the physico-chemical and biochemical phenomena that contribute to the development of flavour **(Gil et al., 1999; Vestergaard et al., 2005)**.

Meat is an important source of animal protein but, at the same time, it includes saturated fatty acids, which makes it a potential cause of different cardiovascular diseases and still little is known about influence of age and sex on these parameters in roe deer muscles **(Cygan-Szczegielniak et al., 2011)**.

Wang et al., (2012) found high salt content could promote the formation of aldehydes from fatty acids.

On the other hand, **Corral et al., (2013)** reported that reducing salt in slow fermented sausages had different influences on the generation of different volatile compounds. The changes of lipid composition, fatty acid composition of phospholipids, and free fatty acids were evaluated by one-way analysis of variance techniques where these measurements were as dependent variables and the processing stage as independent variables.

Several food additives are added in food for their preservation to maintain the freshness of food (antioxidants) or to slow down or stop the growth of microorganisms (preservative agents). Nitrites and nitrates are used as preservative agents in meat. Nitrites give a smoked taste, a pinkish color in the meat and protect the consumers against the risk of bacterial deterioration. Their addition is however very limited as, in high dose, it can have risks on human health and the environment. Nitrites may also combine with secondary or tertiary amines to form N-nitroso derivatives. Certain N-nitroso compounds have been shown to produce cancers in a wide range of laboratory animals. Thus, alternatives of nitrates and nitrites are the object of numerous research studies. Alternatives, such as the addition of vitamins, fruits, chemicals products, natural products containing nitrite or spices, which have similar properties of nitrites, are in evaluation. In fact, spices are considered to have several organoleptic and anti-microbial properties **(Gassara et al., 2015)**.

MATERIAL AND METHODOLOGY

Animals and sample collection

The investigations involved 30 red deer (*Cervus elaphus*) coming from Slovak region. Samples (*semitendinosus* muscle and *triceps brachii* muscle) were collected at the deer slaughter plant from 30 (*Cervus elaphus*) males (<1.5 years). These animals had grazed on summer pasture on the west part of Slovakia. The chill after slaughter started at 6 – 8 °C with a reduction to a deep leg temperature of <2 °C overnight, approximately 9 – 15 h after dressing of the carcass. After deboning the following day, samples were vacuum packed, and stored at 2 – 3 °C for 7 days *post mortem* before being frozen at approximately -20 °C. Samples were thawed at 3 – 4 °C for approximately 15 h and used for all measurements.

Curing

Curing of red deer meat was realized by bringing a concentration of 10% NaCl and 10% Nitrate salt. The ratio of brine and the meat was at 3 : 1 in order to ensure the desired diffusion of salt into each sample. Thus, samples were loaded in the cold brine stored at 4 °C for 7 days. After 7 days cold storage, samples were desalted in lukewarm water for 30 minutes and then put into the smoking chamber, where they were dried at about 24 °C for 6 hours. After sufficient drying, the samples were cold smoke three times in two hours. Thus processed samples were subsequently placed in a climatic chamber where the climatic conditions during storage are gradually adjusted until the temperature has not been reached 15 °C, 75% relative humidity and air flow of 0.3 $m.s^{-1}$.

Fatty acids composition

Extracted lipids were dissolved in 5 mL hexane; 1 mL 2M KOH in methanol was added. The tubes were capped and stirred for 30 min to separate into two phases. The upper phase was analyzed using gas chromatography. A 6890 GC with a Multi Mode injector, a 7683B automatic liquid sampler and flame ionization detection (Agilent Technologies, Palo Alto, CA) were used. Separation was performed with a (60 m × 0.25 mm i.d. × 0.15 μm DB-23) column (Agilent 122-2361). The temperature programme was an initial 50 °C with a 1 min hold, ramp 25 $°C.min^{-1}$ to 175 °C, then 2 $°C.min^{-1}$ to 230 °C with a 5 min hold, then 120 $°C.min^{-1}$ to 245 °C with an 8 min hold. Injector temperature was 250 °C. Carrier gas was H2 with a pressure of 238.96 kPa/2.225 $mL.min^{-1}$. Fatty acid analysis was performed by auto injection of 1 μL of each sample at a split ratio of 1 : 10, constant flow mode, velocity 20.4 $cm.s^{-1}$. The flame ionization detector temperature was 280 °C with H2 35 $mL.min^{-1}$, air 350 $mL.min^{-1}$, and N2 make-up gas flow rates of 30 $mL.min^{-1}$, respectively. The run time for a single sample was 32 min.

Statistical analysis

To determinate the effect of curing on fatty acid profile, each detected fatty acids was analyzed using analyses of variance, the content of each fatty acid was the dependent variable, and the kind of curing was the independent variable. Analysis of variance (Single Factor ANOVA) was used to compare the effects of different curing methods on the fatty acids content. The dependent variable was log10 transformed in the order to meet the assumption of homogeneity of variance. A significance level of $\alpha = 0.05$ was applied to the entire statistical analysis.

The basic idea of Principal component analysis (PCA) is to describe the variation of a set of multivariate data in terms of a set of uncorrelated variables, each of which is a particular linear combination of the original variables. The new variables, namely principal components the total number of which equals the number of the original variables in the studied data, are derived in decreasing order of importance so that, for example, the first principle component accounts for as much as possible of the variation in the original data. The second component is chosen to account for as much as possible in the remaining variation subject to being uncorrelated with the first component, and so on. The usual objective of this type of

analysis is to see whether the first few components account for most of the variation in the original data. If so, they can be used to summarize the data with little loss of information. A reduction in dimensionality is thus achieved which might then be useful in visual interpretation of the data represented by two-dimensional graphics. In order to obtain the web, each variable was transformed so that the group having the highest value for a specific variable was set at 1.0 on the radial scale and values for the other two groups were expressed relative to that. All the computational work, including the graphical presentations, was performed using XLSTAT 2014.5.03 (2014) and Tanagra 1.4.50 (2003) package program.

RESULTS AND DISCUSSION

As shown in Table 1, the percentages and standard deviations of free fatty acids with respect to the total free fatty acids (%) extracted from raw meat, meat cured using NaCl and meat cured using nitrate salt. The main identified fatty acids in all different types of curing were palmitic acid (16 : 0), oleic acid (c18 : 1 cis-9), stearic acid (C18 : 0). This profile doesn´t coincides with that found by other authors in pork meat **(Franco et al., 2002; Johansson et al., 2015)**. In addition, the increase of free fatty acids could also be partially associated with increased activities of acid and neutral lipases due to dehydration and salt diffusion **(Vestergaard et al., 2000)**. These results are consistent with other studies that investigated both free-range and farmed red deer **(Purchas et al., 2010; Triumf et al., 2012)**.

These results are in accordance with **Soriano et al., (2006)** who studied free fatty acids in commercial saucissons made with deer meat.

All the examined samples showed a higher content of SFAs and MUFAs than PUFAs. The individual FAMEs measurement can allow to understand the composition of the fatty acids in the acylglycerols fraction of the examined samples **(Johansson et al., 2015)**.

Paleari et al., (2003) obtained a total saturated fatty acid content of 35.5 – 44%, monounsaturated of 30.3 – 45.7%, and polyunsaturated of 16.2 – 19.6%, respectively, in fat extracted from cured products of deer and wild boar from farms. In the present study, the amounts of saturated acids found were higher; however, the polysaturated acid content was lower.

The resulting *n*-6/*n*-3 PUFA ratio in the muscle samples of red deer showed no variation in different types of curing and was beneficially low within the range of 3.9 : 1 and 4.49 : 1. These results are consistent with other studies that investigated the fatty acid profiles of muscle of red deer and showed higher PUFA contents *n*-6/*n*-3 PUFA **(Polak et al., 2008; Purchas et al., 2010; Triumf et al., 2012)**. According to **Demeyer et al., (1974)** and **Soriano et al., (2006)**, in fermented sausages, there is a tendency to hydrolysis of linoleic, oleic, stearic and palmitic acids, probably because of the specific lipolysis developed by microbial lipases, that is dependent on position and structural conformation of fatty acids in glycerides **(Alford et al., 1971; Soriano et al., 2006)**.

The concentration of free fatty acids in the fat depends on

Table 1 Percentages and standard deviations of free fatty acids with respect to the total free fatty acids (%) extracted from raw meat, meat cured using NaCl and meat cured using nitrate salt (mean ±*SD*).

Fatty acid	Curing method					
	Raw		NaCl		Nitrate	
	SM	***TB***	***SM***	***TB***	***SM***	***TB***
C14 : 0	5.40 ±1.01	4.77 ±0.73	5.85 ±1.34	4.57 ±0.84	5.82 ±1.62	4.42 ±0.77
C14 : 1	1.74 ±0.52	1.36 ±0.81	1.76 ±0.69	1.11 ±0.62	2.44 ±1.31	1.14 ±0.65
C15 : 0	0.67 ±0.21	0.82 ±0.14	0.72 ±0.27	0.77 ±0.18	0.70 ±0.24	0.80 ±0.17
C16 : 0	28.4 2±1.70	24.26 ±1.47	29.12 ±1.88	23.80 ±1.27	27.32 ±1.72	23.73 ±1.33
C16 : 1	9.50 ±2.63	6.93 ±3.86	9.41 ±3.45	6.23 ±3.57	11.63 ±5.39	6.51 ±3.70
C17 : 0	0.68 ±0.15	0.81 ±0.21	0.71 ±0.20	0.79 ±0.23	0.63 ±0.21	0.82 ±0.23
C18 : 0	11.71 ±1.20	17.55 ±3.84	12.12 ±2.44	17.84 ±3.58	10.80 ±2.78	17.77 ±3.79
C18 : 1cis n9	19.12 ±1.18	20.78 ±2.70	19.57 ±0.89	19.93 ±2.67	18.67 ±1.04	20.52 ±2.22
C18 : 2cis n6	6.26 ±0.89	6.78 ±1.27	5.27 ±1.86	7.92 ±2.10	5.53 ±2.75	7.36 ±1.48
C18 : 3 n3	1.57 ±0.27	1.90 ±0.43	1.30 ±0.44	2.01 ±0.57	1.32 ±0.56	1.88 ±0.46
C20 : 4 n6	1.91 ±0.22	2.11 ±0.42	1.18 ±0.44	2.16 ±0.72	1.45 ±0.67	1.97 ±0.51
C20 : 5 n3	0.58 ±0.16	0.39 ±0.18	0.35 ±0.17	0.43 ±0.26	0.36 ±0.17	0.37 ±0.20
PUFA	10.44 ±1.45	11.31 ±2.19	8.29 ±2.92	12.85 ±3.48	8.80 ±4.21	11.96 ±2.52
MUFA	30.55 ±2.61	29.10 ±5.71	30.98 ±3.66	27.30 ±5.77	32.90 ±5.76	28.20 ±5.49
SFA	47.05 ±2.42	48.40 ±5.61	48.87 ±2.77	47.95 ±4.91	45.58 ±5.43	47.73 ±5.16
Σn3/Σn6	0.26 ±0.02	0.25 ±0.04	0.25 ±0.04	0.23 ±0.04	0.24 ±0.03	0.23 ±0.04
Σn6/Σn3	3.90 ±0.29	4.11 ±0.91	4.07 ±0.54	4.44 ±0.84	4.25 ±0.51	4.49 ±0.88

Note: SM – *Semitendinosus* muscle; TB – *Triceps brachii* muscle.

the hydrolytic activity of the lipases, the microbial metabolic processes, and the oxidative reactions that work on the free fatty acids released in the lipolysis. These are directly related to the raw material used to prepare the sausages, ingredients, additives and spices added, and the production process **(Soriano et al., 2006)**.

No significant differences were found between the different curing methods for *Semitendinosus* muscle (ANOVA, p >0.05, F – 0.003, F crit. – 3.041) and *Triceps brachii* muscle (ANOVA, p >0.05, F – 0.05, F crit. – 3.01).

The results of the PC analysis are presented in Table 2. The first three PC explain 100% of total variation for percentage fatty acids content. **Laville et al., (1996)** found the first ten PCs analysing 76 morphometric variables from young Charolais bull carcass explained 80% of the total variability of those measurements. However, in rabbits **Hernández et al., (2000)** reported the four first PCs for meat quality explained 62% of the total variation. They analysed meat quality using 23 variables, including pH, meat colour, WHC, cooking loss, fatty acid composition and sensory parameters.

Cañeque (2004) found the first five PCs analysing 20 variables from light lambs explained 77% of total variation. **Šnirc et al., (2016)** reported the first five PC explained more than 85% of total variation for pH, chemical and technological parameters and higher level 86% for textural parameters for meat from farmed red deer and pastured red deer.

Figure 1 shows the loading plot of the measurements of fatty acids content on the first two PCs. The measurements and PCs are interpreted according to the correlations between each parameters and each PC, thus measurements close to each other are positively correlated, measurements separated 180° are negatively correlated, whereas if they are separated by 90° they are independent. The loading plot displays that these measurements are placed far from the origin of the first PC and near each other indicating the high correlation among them (Figure 1).

Table 2 Results from principal component analysis for fatty acids content.

	F1	F2	F3
Eigenvalue	2.9797	0.0164	0.0039
Variability (%)	99.3238	0.5460	0.1301
Cumulative (%)	99.3238	99.8699	100.0000

Note: F1 - Principal component 1; F2 - Principal component 2; F3 - Principal component 3.

The results of present study are in accordance with **Gladwin et al., (2005)** who studied free fatty acids in commercial saucissons made with deer meat. The amounts of these majority acids are similar to those found in our work. The use of salt in production of fermented meat is without alternatives, as it affects fundamental processes in meat conditioning, on microbial performance, hygiene, shelf life, flavour and texture **(Desmond, 2006)**.

Chorizos and saucissons made with deer meat and commercialised in the Spanish market had a higher fat content than those prepared with wild boar meat, and a further two factors that may be differentiating were the protein nitrogen and phosphorus content, which were higher in dry sausages made with wild boar. Chorizos, made with deer or wild boar meat, had higher percentages of polyunsaturated free fatty acids, linoleic and linolenic acids, and lower percentages of the monounsaturated 11-eicosenoic acid, than had the saucissons **(Soriano et al., 2006)**.

Paleari et al., (2006) recorded that cured, fermented and dried products of wild boar and horsemeat showed reduced values of saturated fatty acids (SFA). The products from wild boar, goat and beef had higher values of

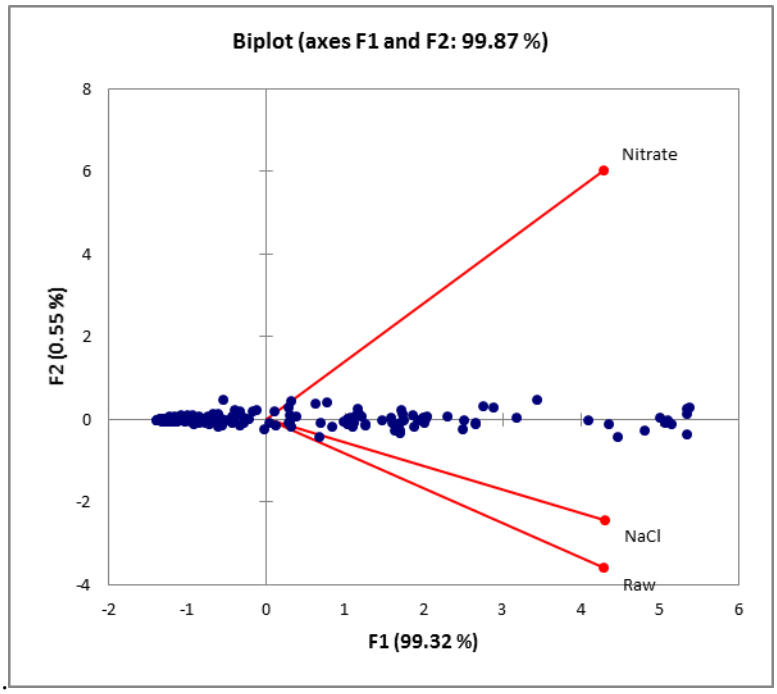

Figure 1 Plot of the first two PC loading vectors. The labels correspond to sample notation given in the Table 1

monounsaturated fatty acids (MUFA). The polyunsaturated fatty acids (PUFA), known to play an irreplaceable dietary role, were found to be highest in the horsemeat product, at an intermediate level in the final product of deer and extremely reduced in the beef product.

CONCLUSION

In this study the effect of different curing methods on the fatty acid composition was evaluated.

The kind of curing salt aren´t significantly affected the fatty acid compounds formation in dry-cured deer meat. The fatty acid compounds were selected based on their presence and contribution of dry-cured meat products.

The different curing agents (sodium chloride and nitrate) were no effect on fatty acids content in dry cured meat products prepared from deer meat muscles. The present study revealed that game meat can function as a good source of bioactive compounds that are essential for human nutrition.

Further research into the relationship between different types of curing on fatty acids profile is needed to determine which compounds could be used as markers of sausage quality.

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