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EFECT OF SMOKING ON THE ACCUMULATION OF POLYCYCLIC AROMATIC HIDROCARBONS, IN M. LONGISIMUS DORSI FROM PIGS AND POSSIBILITIES FOR REDUCING THEIR CONTENT

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This study deals with the effect of smoking process and the accumulation of toxic polycyclic aromatic hydrocarbons in Musculus Longissimus dorsi from pigs and studies the possibilities to reduce their content.

The studies were conducted on two samples boiled-smoked fillet – one salted with brine and added protein coating, whereas the second one salted with brine and added ascorbic acid.

Qualitative identification and quantification of polycyclic aromatic hydrocarbons is done by gas hromotograph Hewlett Packard 5890 with LG 85 B spectrophotometric detector. It was found that the use of protein coatings, significantly reduces the amount of polycyclic aromatic hydrocarbons in the smoking of Musculus Longissimus dorsi from pigs. The addition of ascorbic acid in conjunction with protein salting coatings technology represents a significant option for reducing the content of the benzo(a)pyrene , which contributes to the safety in the final products. In studies of the samples of proteins and ascorbic acid, the inner layers i.e the muscle tissue was found to contain significantly less amount of benzo(a)pyrene (1,75 ± 0,06 μ/kg) compared with the values found in the surface layers (2,31 ± 0,02 μg/kg).

Key words: meat, polycyclic aromatic hydrocarbons (PAH), benzo (a) pyrene (BaP).

У статті наведені дані щодо впливу процесу копчення на накопичення токсичних поліциклічних ароматичних вуглеводнів у найдовшому м'язі спини свиней а також дослідження щодо можливості зниження вмісту цих речовин.

Дослідження проводилися на двох зразках варено-копченого філе: один з білковим покриттям та оброблений сольовим розсолом, другий – з білковим покриттям, оброблений сольовим розсолом в який додали аскорбінову кислоту.

Якісну ідентифікацію та кількісне визначення поліциклічних ароматичних вуглеводнів здійснювали за допомогою газового хроматографу Hewlett Packard 5890 із спектрофотометричним детектором LG-85-B. Було виявлено, що використання білкових покриттів, значно знижує кількість поліциклічних ароматичних вуглеводнів в копчених м'язах свинини Longissimus dorsi. Додавання аскорбінової кислоти у поєднанні з білковим покриттям в технології засолювання представляє собою важливий варіант для зниження вмісту бензо(а)пірена, який забезпечує безпеку кінцевих продуктів. Дослідження зразків – внутрішніх шарів м'язової тканини дозволило встановити, що вони містять значно меншу кількість бензо(а)пірену (1,75 ± 0,06 мкг/кг) порівняно зі значеннями в поверхневих шарах (2,31 ± 0,02 мкг/кг).

Ключові слова: м'ясо, поліциклічні ароматичні вуглеводні (ПАУ), бензо(а)пірен (Бап).

Introduction

In recent years one of the highest priorities in the meat industry is developing new technologies to ensure the production of high quality and safe meat products.

Epidemiological studies have shown that during the processes heating and smoking meat, compounds are formed with genotoxic effect for humans (Jira W., 2005; Chao A, et.al., 2005). One group of these substances, such as polycyclic aromatic hydrocarbons are formed during the traditionally used regimes of smoking meat products and have high mutagenic and carcinogenic potential (Falco, G., et al., 2003; Hitzel, A. et al., 2013). The study of these compounds is a priority problem in the world, primarily due to their toxicity. One of the first identified compounds from the group of polycyclic aromatic hydrocarbons (PAH) is benzo(a)pyrene, which has carcinogenic effects and is regarded as an indicator of contamination of smoked meat products (Farhadian, A., et al., 2012; Higginbotham, S., 1993).

The content of benzo(a)pyrene in smoked products significantly depends on the technology of smoking (Hitzel, A. et al., 2013; Pavanello S., 2008). In conventional smoking constituents of smoke, for the most part are found in the surface layer of the product.

Quantity and variety of these compounds depends on many factors. Especially important are the methods and processing conditions (Farhadian, A., et al., 2012; Janoszka B., 2011). In most traditional regimes of smoking meat products, PAHs are formed in an amount ranging from several hundred g/kg to traces (Farhadian, A., et al., 2012; Nawrot, PS, 1999). Applying hot smoking is however, associated with raising the temperature to 95 °C in the smoking chamber. These temperatures are primarily achieved in the surface layers of the products subjected to hot smoking. The purpose of this paperwork is to study the influence of smoking on the content of polycyclic aromatic hydrocarbons with toxic impact in Musculus Longissimus dorsi from pigs and to examine the technological possibility to reduce their content by using a protein coating and the addition of ascorbic acid.

Material and methods

Studies were conducted with boiled-smoked fillet – m.Longissimus dorsi from pigs. For the production of the samples, chilled pork halves were used, m.Longissimus dorsi, and meat pieces are processed through wet salting. Prepare two test samples – salted with brine having a concentration 14 °Be, with the addition of protein sample preparation and salted with brine having a concentration 14 °Be, and addition of ascorbic acid (500 mg/kg meat mass). The proteins consist of protein hydrolyzate, and hydrocolloids. They were added in an amount of 7 kg/100 l brine. In the same time control samples of fillets were prepared by traditional technology, which uses wet salting only with brine at a concentration of 14 °Be. Heat treatment of tested samples of pork fillet – m.Longissimus dorsi is done in meat smoking chamber. Originally fillets are dried and smoked. The samples were then boiled at a temperature of (76-80) °C until the temperature at the center is 72 °C, and then are cooled and stored at 4 °C until analysis begins.

For qualitative identification and quantification of polyciclic aromatic hydrocarbons, tested samples have been processed in the following manner:

200 g. of each sample is grounded into a mixer at 200 g, then 25 g were weighed and placed into a cellulose ampoule. 200 cm³ of acetone was added and extracted for 5 h at room temperature. The resulting extract evaporates to dryness in a rotary evaporator at a water temperature of 40 ºC. The resulting dry residue is then hydrolysed with 100 cm³ solution of KOH in ethanol for 5 min at a water temperature of 60 ºC. The solution was then transferred in a separatory funnel, and to this 100 cm³ of cyclohexane was added and 100 cm³ of distilled water at 60 ºC, stirred and left for decantation. If the emulsion is formed, 10 cm³ of ethanol is added. The aqueous phase was separated, and the organic phase was filtered through sodium sulfate bezvozden. The resulting filtrate was evaporated to dryness in a rotary evaporator at a water temperature of 40 ºC. The resulting dry residue was then dissolved by 2 cm³ of a mixture of methanol and tetrahydrofuran in a ratio of 1:1.

Six µl of this solution was injected into a Hewlett Packard 5890 gas hromotograph in LG 85 B spectrophotometric detector at $\lambda = 254$ nm. Analyses were performed under the following conditions for hromotography: column SC – CAB 25 x φ 2,6 mm; mobile phase consisting of a mixture of water and acetonitrile at the below described conditions :

Min.	**% CH_3CN**	**% H_2O**
10	50	50
3,0	50	50
7,0	100	0
25,0	100	0
5,0	50	50

In order to identify the peaks, obtained after injection of the sample into the liquid chromatography, the time of retention of indicators and the internal additive method are used. The amount of polycyclic aromatic hydrocarbons identified is determined by the external standard method.

The results obtained are processed mathematically – statisticaly (Georgieva, et.al., 1989).

Results and discussion

The experimental results of studies on the composition and quantity of polycyclic aromatic hydrocarbons (PAHs) in samples of boiled-smoked pork fillet using protein coatings and composite mixture are presented in Table 1. In all studied samples m.Longissimus dorsi, a total of 15 different compounds from the group of polycyclic aromatic hydrocarbons are defined quantitatively and identified qualitatively.

From the obtained results it can be noticed that in the control samples significantly higher levels of a variety of surfactants as compared with the test samples (see Table 1) are accumulated. We believe that the probable reason for this is the presence of surface protein coverage in test samples, which contributes to the formation of compacted layer on the surface that hinders the penetration of polycyclic aromatic hydrocarbons from the smoke for smoking.

Table 1 – Changes in the amount of PAHs (µg / kg) in samples cooked smoked pork loin (m.Longissimus dorsi), produced with protein preparation (BP) with and without the addition of ascorbic acid (AC)

Name	m.Longissimus dorsi (control samples)	m.Longissimus dorsi (experimental samples with PC)	m.Longissimus dorsi (experimental samples with PC+AA)
1. Benzo/a/-antracene /BaA/	11,14 ± 0,10	9,03 ± 0,07	5,09 ± 0,06
2. Chrysene /CHR/	10,22 ± 0,05	6,51 ± 0,07	4,78 ± 0,05
3. Cyclopenta/c,d/-pyrene /CPP/	12,53 ± 0,11	7,80 ± 0,10	6,22 ± 0,08
4. Methyl- chrysene. /5MC/	8,51 ± 0,09	6,17 ± 0,05	3,64 ± 0,03
5. Benzo/b/-fluoranthene /BbF/	9,32 ± 0,11	7,70 ± 0,04	5,70 ± 0,09
6. Benzo /κ/fluoranthene /BkF/	7,95 ± 0,08	5,47 ± 0,06	4,13 ± 0,07
7. Benzo/j/-fluoranthene /BjF/	8,07 ± 0,04	6,10 ± 0,03	3,86 ± 0,04
8. Benzo /e/ pyrene /BeP/,	11,10 ± 0,06	8,62 ± 0,04	6,35 ± 0,07
9. Dibenso /a,h/-anthracene /DhA/	10,12 ± 0,11	7,75 ± 0,05	5,59 ± 0,08
10. Indeno /1,2,3-c,d/-pyrene /IcP/	6,08 ± 0,04	4,59 ± 0,05	2,14 ± 0,03
11. Benzo /g,h,i/-perilene /BgP/	9,76 ± 0,07	6,92 ± 0,08	4,80 ± 0,09
12. Dibenzo/a,I/-pyrene /DIP/	8,41 ± 0,06	5,70 ± 0,03	4,29 ± 0,05
13. Dibenzo /a,e/-pyrene /DeP/	7,00 ± 0,01	6,38 ± 0,03	4,08 ± 0,01
14. Dibenzo /a,i/-pyrene /DiP/	7,34 ± 0,04	6,05 ± 0,05	4,65 ± 0,03
15. Dibenzo /a,h/-pyrene /DhP/	8,17 ± 0,04	6,83 ± 0,07	3,20 ± 0,02

Using protein preparation and the addition of ascorbic acid in the technological processing of m. Longissimus dorsi leads to significant reduce of the amount of dibenzo /a, i / – pyrene (DiP) (Table 1) for which there is evidence that it has a strong carcinogenic potential (Higginbotham et.al., 1993).

The results obtained from the analyses to determine the effect of the use of the protein coating, and the protein layer + ascorbic acid on the contents of benzo(a)piren in ready fillets showed accumulation of large amounts of this compound in the control samples, as compared to test samples produced with the addition of the protein preparation (Table 2) .

Table 2 – Content of benzo (a) piren (µg / kg) in samples cooked smoked pork loin (m.Longissimus dorsi), produced a protein preparation with and without the addition of ascorbic acid

Samples	BaP Content (µg/kg)
m.Longissimus dorsi (control samples):	
Surface lauer	7,48 ± 0,05
Deep lauer	6,72 ± 0,07
m.Longissimus dorsi (test samples with a protein coating):	
Surface lauer	5,19 ± 0,03
Deep lauer	4,75 ± 0,05
m.Longissimus dorsi (test samples with a protein lauer +ascorbic acid):	
Surface lauer	2,31 ± 0,02
Deep lauer	1,75 ± 0,06

The data in Table 2 show that the greatest amount of hydrocarbons with the greatest carcinogenic potential for the consumers' health as benzo(a)pyrene, is contained in the surface layer of the control fillets, followed by its accumulation in the deep layer of muscle tissue. At substantially lower amounts benzo (a) is accumulated in the test sample fillets i.e in the sample with a protein coating and ascorbic acid, the least amount of BaP was found in the surface layer and the depth of the muscle (Table 2).

When studying the inner muscle layer, which are not in direct contact with the smoke for smoking of fillets, considerably less amount of benzo(a)pyrene (1,75 ± 0,06 μg/kg) was found when compared with the values that were detected in the surface layers (2,31 ± 0,02 μg/kg), (Table 2). We believe that this significant reduction in the amount of one of the most toxic compounds from the group of PAHs is due, on the one hand, to the addition of ascorbic acid. On the other hand, protein coating is formed on the surface of the test sample of muscle tissues m.Longissimus dorsi. This hinders the diffusion of the PAHs from the surface layers to the inside. This seems to have additional impact on the most significant reduction of its content in the final product (Table 2).

Conclusions

Using protein coating significantly reduces the amount of polycyclic aromatic hydrocarbons with toxic and carcinogen effect. The accumulation is in the final products of the smoking process of Musculus Longissimus dorsi from pigs. The addition of ascorbic acid when salting with protein coating represents a technological option for significant reducing of the content of benzo(a)pyrene, which contributes to the safety in the final products.

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