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Influence of Casing Types and Cold Storage on Polycyclic Aromatic Hydro Carbons (PAHs) in Smoked Beef Sausage

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Abstract

The sheep sausages casing and cellulose casings smoked in indirect traditional smokehouses were studied in order to assess the influence of smoking conditions on the PAH contents. Dependency on hot smoking conditions (with indirect smoking), using two types of casing (sheep casing and cellulose casings).All smoked samples are storage at 4°C For 90 days. Smoked sausages samples by indirect technique, using smoke from an external smoke generator were assessed. The total Polycyclic aromatic hydrocarbons (PAHs) appeared in study, divided to three group; the first group was low molecular weight PAHs recorded very high value in two types of beef sausage (2209.2285 µg /Kg and 598.865 µg /Kg); The second group (PAH4) recorded very low content in two types of beef sausage (0.0007 µg /Kg). Benzo(a) pyrene (BaP) levels were below the limit of quantification (0.0002µg/kg) appeared in products. The third group was heavy PAHs recorded (481.7937 µg /Kg and 0.0009 µg /Kg) in sheep and cellulose; respectivly. Cold storage of samples caused significant decrement of (PAHS). Based on this results it could be concluded that smoked sausage samples by indirect technique and used cellulose casing caused significant decrement of (PAHs). As compared with indirect technique and sheep casing.

Introduction

Smoking is one of the oldest technologies for the conservation of meat and meat products, and is defined as the process of penetrating meat products with volatiles resulting from the thermal destruction of wood (Toth, 1982). Wood consists of approximately 50% cellulose, 25% hemicellulose and 25% lignin (Toth and Potthast, 1984). Lignin from softwood differs structurally from that of hardwood. The pyrolysis of lignin phenolic substances produced, are of considerable importance for the organoleptic properties of smoked meat products (Bratzler, *et al.,* 1969; Kjallstrand and Petersson, 2001) and show antimicrobial (Davidson and Branden, 1981) and antioxidative effect (Toth, 1982; Pöhlmann *et al.,* 2012). However, as an undesired consequence of smoking, polycyclic aromatic hydrocarbons (PAH) are generated due to the incomplete combustion of wood. About 660 different compounds belong to the PAH group, some of there show carcinogenic properties (IARC, 2010). The carcinogenic properties the PAH contents in food should be ‘‘as low as reasonably achievable’’ (SCF, 2002). The PAH contamination of foods can also be reduced by replacing direct smoking (smoke produced in the smoking chamber) with indirect smoking where the smoke is obtained

by an external smoke generator, operated under controlled conditions, namely the combustion process and the smoke production temperature (Duedahlolesen *et al.,* 2006). Is also important for the food industry that the smoke generation at a given temperature be done without the wood catching fire (ReySalgueiro *et al.,* 2004; Simko, 1991; Dennis *et al.,* 1991). Lowering the temperature of smoke formation to 300-400 °C combined with the use of filters, the PAH content of smoke can be decreased by about 90% (Sikorski, 2005). Most products made of natural casings come out with a curve after filling and cooking. Artificial casings are now made with collagen, cellulose and plastic materials to suit a wide range of applications (Abdolghafour, and Saghir, 2014) . In all conditions tested, about 70% - 92% of total PAHs were found in the casing when products were filled in natural casings, irrespective of the smoking regime processed. Maximal concentrations of PAHs usually occur at the product surface (Marques, *et al.,* 2011). Many of PAHs had been demonstrated to be carcinogenic for experimental animals and they are also supposed to have a significant impact on the disease burden caused by various cancers in human populations (Phillips, 1999). Joint Expert Committee on Food in the European Union reported that two maximum levels for PAH in smoked meat products are existing: A maximal level for benzo[a]pyrene (BaP) (5 µg/kg) and a maximal level for the sum content of the four PAH compounds BaP, chrysene (CHR), benzo[a]anthracene (BaA), and benzo[b]fluoranthene (BbF) (PAH4) of 30 µg/kg. In September 2014 these maximal levels were lowered to 2 µg/kg (BaP) and 12 µg/kg (PAH4); respectively (Commission Regulation (EC) No 1881/2006) amended by Commission Regulation (EU) No 835/2011).

The main objective of the studies was to analyses the effect of smoking method, types of casing and cold storage time on the PAH contents in zero time and after cold storage for 90day (BaP and15PAH) the type of casing (cellulose casing and sheep casing), using (Sawdust wood).

Materials and Methods

Materials

Meat

12kg of fresh lean beef from young cow (1 years old) were obtained from Assiut slaughter house during November 2014. Visible fat and connective tissue were manually eliminated (Martinez, 2009). The lean beef samples were minced using meat mincer and was used for processing of beef Sausage.

Local market sausage

6.0 kg of cooked beef sausage purchased from Hyper One in Sixth of October City local market during November 2014. The cooked beef sausage samples. Stored in a deep freezer at -20˚C in the laboratory until taken to analysis.

Laboratory Beef sausages formula

Basal beef sausage formulae presented included minced meat 68gm, salt 1.8gm, garlic 0.53gm, mixed spices 1gm, Fat 15gm, Sodium nitrite 0.01gm, Tripolyphosphates 0.3gm, Ascorbates 0.3gm, fillers 1.5 gm and iced water 11.56ml Egyptian Standard for sausage (1991).

Cooking of beef sausage

The sausage was cooked in hot water at 80 °C until the temperature of the geometric centre reached 75 °C for 20 min. The cooked sausages were used to analyze the physico-chemical, textural, and sensory properties on that day. (Abdolghafour and Saghir 2014).

Smoking of beef sausage

After cooking the sausages made in the laboratory taken two types of cooked beef sausage by deferent types of casings namely sausages with cellulose casings local market sausage and sheep casings laboratory sausage are smoked in hot Smoking using (indirect smoking). The temperature of smoke at 50-60°C for 60 min in smokehouse by used Sawdust beech and use of filters of glass wool the content of moisture beech 22.02 %. Smoked product were placed in dishes of cork and taken to the laboratory for analysis and stored at 4 °C in refrigerator for 3months. Zero time 1, 2 and 3 months for analyses during storage (Skrbic, *et al.,* 2014; Huang, *et al.,* 2001).

Analytical Methods

Determination of Polycyclic Aromatic Hydrocarbons (PAH)

Extraction: The PAH.

Soxhlet extraction and clean-up

Prior to extraction, silica gel was activated as described by (Ikechukwu *et al.,* 2012) by oven-drying for 24 hours at 130 C. Concentrated H_2SO_4 acid was then added to the silica gel (1:1v/v) and the mixture shaken vigorously. The mixture was then stored at room temperature prior to analysis.

Five grams of the beef sausage sample was weighed and homogenized with 5g of anhydrous sodium sulphate in a laboratory mortar until a complete homogenate was obtained. The extraction was carried out using a Soxhlet extractor apparatus consisting of a 250cm^3 round bottomed flask, condenser and an extractor tube, seated in a temperature controlled heating mantle. Rotary evaporator was used to evaporate the extract to the desired concentration. The homogenate was carefully transferred into the extraction thimble placed in the extraction chamber of a Soxhlet extraction unit. The extraction was carried out as recommended by USEPA 3540 method (USEPA, 1994), using 150 cm^3 dichloromethane for 16 hours. The extract was concentrated to 2 cm^3 using a rotary evaporator in a water bath preset to a temperature of 35^oC and was stored in an amber bottle and kept in a refrigerator to avoid oxidation of the extract prior to clean up.

Sample purification

The extracted samples were purified by passing them through a silica gel column chromatography, as described by Grimmer and Boehnke (1975) prepared by loading 10 g of activated silica gel onto a chromatographic column (1cm^3 internal diameter) to about 5cm^3. This was topped with 1cm^3 of anhydrous Na_2SO_4. It was then conditioned with dichloromethane. 2 cm^3 of the concentrated extract was loaded and eluted with 20 cm^3 of dichloromethane. The eluent was re-concentrated to dryness using a rotary evaporator (30 °C) with nitrogen stream (35 °C; 7−9 psi) and dissolving the dried concentrate in 1 mL of acetonitrile and filter then transferred

to 2 ml amber screw-cap vials to avoid oxidation and analyzed using GC-mas.

Gas Chromatography – Mass Spectrometry (GC-MS). Gas chromatography was performed in an GC-MS (7890A-5975B) capillary chromatograph equipped with a flame ionization detector and a DB-5ms quartz capillary column (30 m .0.250 mm; phase layer, 0.25 µm). The ether extracts were analyzed using a column temperature programmed in the following mode: isotherm at 40°C for 2 min; then4°C at min. to 50°C for 3 min. then10 °C/min. to 150°C for 3min, then10°C/ min to 220°C for 6 min.then 15°C / min to 280°C for 10 min. The injector and detector temperatures were 260°C; carrier gas rate,. 5 ml/min for 10.9 min. then 1ml/ min per min. to 1 ml / min for 30 min.

Results and Discussion

Effect of indirect smoke on polycyclic Aromatic Hydrocarbons (PAH) content in beef sausages

Data presented in Table (1) showed that the influence of indirect smoke on the formation of polycyclic aromatic hydrocarbons PAHs. The low temperature during the smoking process. Appe ared three ring from PAHs in study.The first studied group was low molecular weight PAHs such as acenaphthene, cenap hthylene, Naphthalene, and Pyren recorded very high value (319.109 µg/kg, 54.622 µg/kg 1359.307 µg/kg, and 76.190 µg/kg); respectively. Such data agree with (Silva, *et al.,* 2011) findings who studied Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs). (Silva, *et al.,* 2011) found that The higher level of low molecular weight PAHs such as acenaphthene, acenaphthylene, Naphthalene. Which are not regarded as very carcinogenic; Second group in study PAH4 recorded very low(0.0007 µg/kg) in two types of beef sausage such as Benzo(a)pyrene, Chysene, Benzo (a) Anthracene, Benzo (b) flouranthene, they were very carcinog enic. The United States Environmental Protection Agency (EPA, 2002) classified the total PAH for two group the first group PAH4 (benzo[a] pyrene, benzo [a] anthracene, chry sene, benzo[b]fluoranthene) probable human carcinogens. and Second group PAHs (naphthalene, acenaphthylene, acenaphthene, pyrene) are not regarded as very carcinogenic (Bostrom *et al.,* 2002) reported that The International Agency for Research on Cancer (IARC) classified BaP as carcinogenic to humans (group 1); other PAHs such as dibenzo [a,h] anthracene (DahA), as probably carcinogenic to humans (group2A); and other PAHs, such as naphthalene (NaP), Benzo [a]anthracene (BaA), Chrysene (Chr), benzo [b] Fluor anthene (BbF), Benzo[j] Fluoranthene (BjF) and indeno[1,2,3-cd]pyrene (Ind) as possibly carcinogenic to humans. The content of Benzo(a)pyrene in these study (0.0002 µg/kg). Commission Regulation (EC) No 1881/2006) reported that A maximal level for benzo[a] pyrene (BaP) (5 µg/kg) and a maximum level for the sum content of the PAH4 compounds BaP, chrysene (CHR), benzo[a]anthracene (BaA), and benzo[b]fluoranthene (BbF) (PAH4) of 30 µg/kg. The level of B (a) P found in the indirect smoke

samples by different casings was very low than the European regulatory maximal level for smoked meat and fishes (Regulation (EC) No 208/2005). The results revealed that the sausages samples smoked by the indirect smoke methods did not constitute a health risk, as the levels of the benzo (a) pyrene was below or lower than maximal levels regulated by the European Commission. In most of the smoked susages studied, benzo (a) pyrene was not detected which agrees with (Muthumbi *et al.,* 2003;Mihalca, *et al.,*2011) who found that Products smoked by an indirect technique using external smoke generators had low PAH and BaP levels that are well below the maximal level of 5.0 mg/kg. (Garcia and Simal, 2005) reported that the levels of PAH in smoke depends on heat source, temperature. (Silva, *et al.,* 2011) found that The PAHs levels were found to vary with the heat source. (Pöhlmann, *et al.,* 2012) reported that the low smoke generation temperatures is a promising approach to lower PAH contents. Which agree with. (Sttumpe-Viksna *et al.,* 2008) who reported that smoking procedure (direct/indirect). Under both smoking styles, benzo[a]pyrene and the PAH4 group did not exceed the current limits of 5 µg/kg which did not represent any significant risk to consumers' health. (Karl and Leinemann1996), asserted that traditional direct smoking, it to higher PAH content than indirect smoking, which used a separate chamber for smoke generation. But different in content of Pyrene in the sheep casing was (476.190 µg/kg) and cellulose casing was (0.0002 µg/kg). (Skrbic, *et al.,* 2014) found that traces of pyrene were detected only in indirect smoke samples. (Rey *et al.,* 2004); The third group in the current study was heavy PAHs recorded (481.7937 µg /Kg and 0.0009 µg /Kg) respectively. Which agrees with (Gomes, *et al.,* 2013) reported that collagen casing led to a significant reduction ($p < 0.05$) in total PAHs content, of about 3 times, when compared to samples stuffed in hog casing.

Table 1. PAHs contents found in (smoked sausage) by indirect smoking method and different casings in zero tim:

No	PAH	Sheep casing	cellulose casing
		(Mg/ kg)	(Mg/kg)
1	Acenaphthene	319.109	176.252
2	Acenapthylene	54.622	0.0001
3	Napthalene	1359.307	0.0001
4	Flourene	0.0002	51.364
5	Anthracene	0.0001	0.0001
6	Phenathrene	ND	ND
7	Flouranthene	0.0002	371.249
8	Pyren	476.190	0.0002
9	Benzo(a)pyrene	0.0002	0.0002
10	Chysene	0.0002	0.0002
11	Benzo(a)Anthracene	0.0001	0.0001
12	Benzo(b)flouranthene	0.0002	0.0002
13	Benzo(K)flouranthene	481.793	0.0002
14	Dibenzo(a,h)Anthracene	0.0003	0.0003
15	Indeno(1,2,3-c,d)pyrene	0.0004	0.0004
16	Benzo(ghi)pyrle	ND	ND
Light PAHs		2209.2285	598.8655
PAH4		0.0007	0.0007
Heavy PAHs		481.7937	0.0009
Total PAHs		2691.0229	598.8671

Effect of casings types on the content of polycyclic aromatic hydrocarpons

Data Presented in Table (1) showed the influence of the casing types on the content of polycyclic aromatic hydrocapons (PAHs). The total concentration of the PAHs in the sausage preferred by sheep casing was least recorded of (2691.0229 µg/kg), while cellulose sausages recorded (598.8671 µg/kg) respectively. The data agree with (Jira, *et al.,* 2013), who found that the sausages with the sheep casings had higher PAH contents than the peeled cellulose sausages. (MANEA, *et al.,* 2015) Semi-smoked sausage allows the absorption of large quantities of PAH from smoke, because the section has a small diameter and have natural casings from sheep. (Gomes, *et al.,* 2013; Garcia-Falcon, and Simal-Gándara 2005) reported that the higher contributions of collagen casing to total PAHs level found in whole product (5–21%) when compared to those found in hog casing (6–10%), showing higher permeability in the latter. In this study Most PAHs were very low in smoked sausage such as fluorene(FLR), anthracene (ANT), fluoranthene (FLT), Benzo (a)pyrene (BaP), chrysene (CHR), benzo[a]anthracene (BaA), benzo [b] fluoranthene (BbF), dibenzo[a,h] anthracene (DhA) and indeno [1,2,3-cd] pyrene- (IcP) for example as was very low the concentration of total PAHs in these compounds(0.00 019µg / kg) and some compounds not detected in smoked sausage such as Phenathrene and Benzo(ghi)pyrle in the two types of casings. Such data agree (Jira*, et al.,* 2013*)* reported that the selection of a cellulose casing is a reasonable approach for reducing the PAH contents in hot smoked sausages.

Effect of cold storage time on the content polycyclic Aromatic hydrocarbons:

Table 2. The effect of cold storage time at 4 °C for 90 days on polycyclic Aromatic hydrocarbons is out lined in Table 2.

No.	PAHs	Sheep sausage casing after storage at 4°C for 90 day	cellulose sausage casing After storage at 4 °C for 90 day
		(Mg/ kg)	(Mg/kg)
1	Acenaphthene	<0.0001	< 0.0001
2	Acenapthylene	< 0.0001	< 0.0001
3	Napthalene	< 0.0001	< 0.0001
4	Flourene	< 0.0002	< 0.0002
5	Anthracene	< 0.0001	< 0.0001
6	Phenathrene	ND	ND
7	Flouranthene	< 0.0002	< 0.0002
8	Pyrene	< 0.0002	< 0.0002
9	Benzo(a)pyrene	< 0.0002	< 0.0002
10	Chysene	< 0.0002	< 0.0002
11	Benzo(a)Anthracene	< 0.0001	< 0.0001
12	Benzo(b)flouranthene	< 0.0002	< 0.0002
13	Benzo(K)flouranthene	< 0.0002	< 0.0002
14	Dibenzo(a,h)Anthracene	< 0.0003	< 0.0003
15	Indeno(1,2,3-c,d)pyrene	< 0.0004	< 0.0004
16	Benzo(ghi)pyrle	ND	ND
Light PAHs		0.001	0.001
PAH4		0.0007	0.0007
Heavy PAHs		0.0009	0.0009
Total PAH		0.0026	0.0026

Data Presented in Table (2) illuotrated that. Smoked sausages samples in two deferent casings showed reduced in low molecular weight PAHs values after storage time. The content of low molecular weight PAHs in smoked sausage by sheep casing and cellulose casings were lower during cold storage (0.0007µg/kg). such as acenaphthene (ACE 0.0001µg/kg), acenaph thylene-(ACY0.0001µg/kg), naphthalene (NAP 0.0001µg/kg), pyrene (PYR 0.0002µg/kg) ; respectively .The PAH4 components staying almost unchanged during storage such as benzo[a] pyrene (BaP0.0002 µg/kg), chrysene(CHR 0.0002 µg/kg) benzo[a] anthracene (BaA 0.0001 µg/kg), benzo [b]fluoranthene (BbF0.0002µg /kg). The heavy PAHs reduced in benzo[k]fluor anthene (Bkf 0.0002µg/kg)in sheep sausage, and other compounds were unchanged during storage such as dibenzo [a,h] anthracene (DhA 0.0003µg/kg), indeno [1,2,3-cd] pyrene (IcP0.0004µg/kg), fluorine(FLR0.0002µg/kg), fluoranthen e (FLT0.0002µg/kg), anthracene (ANT0.0001µg/kg), phenanthre ne (PHE ND) and benzo[g,h,i]perylene-(BgP NDµg/kg). In smoked sausage by cellulose casings giving the same results reduced PAHs in the samples at (0.0026µg/kg). such result data agree with (Marques, *et al.,* 2011) who reported that PAH8 had decreased effect due to cold storage conditions for 90 day at 4°C. The mean PAH4 (4.5 µg kg-1) and PAH8 (4.65 µg kg-^{1}) values found in final products decreased 2.5 and 1.98 times, respectively after cold storage. Maximal concentrations of PAHs usually occured at the product surface. (Roseiro, *et al.,* 2011). Reported that concerning the occurrence of PAHs in the smoked products, it had been proven that PAHs accumulated mainly on the product's surface of the product. (Marques, *et al.,* 2011; Garcia-Falcón and Simal-Gandara, 2005; Djinovic *et al.,* 2008) Reported that different PAHs adsorption capacities by the products surface, the level of PAHs was analyzed in both the casing and inner portions in all conditions tested, about 70% - 92% of total PAHs were found in the casing. (Jira, *et al .,* 2013) reported that in spite of the higher weight losses of sausages the PAH contents in these sausages were lower. (Simko, 1991, Dennis *et al.,* 1991; Simko, 2002) Found that the highest content of PAHs in smoked products appeared immediately after finishing the smoking operation, decreasing then over time. (Simko, 2005). Found that after 31 days the less constant of BaP in the salami. The average BaP content in the final product was lowered only by 17% compared with the sample analyzed immediately after smoking.

In conclusion

Smoked products by an indirect technique using external smoke generators recorded very high content in low molecular weight compounds (PAHs); The low molecular weight of PAHs are not regarded as very carcinogenic Considering the PAHs from the EPA priority list. Reduced generation temperature gave high levels of volatile PAHs into the surface of products so generation temperature should not be lowered below 500 °C. The industrial smokehouse

with traces of pyrene was detected only in indirect samples. The PAH4 and BaP levels reduced indirect smoke were very low in smoked sausage samples. The use of cellulose casings contributing decisively to reduced the total PAHs content in the smoked sausages compared to sheep casings, So traditional meat products processing mandatory include the use of cellulose casings, in indirect smoking regimes are applied. However, for safety reasons, the removal of any type of casings prior to consumption is recommended. According to 2008 EFSA recommendations, detected contamination levels in traditional processing procedures did not represent any considerable risk to consumers' health. The content of total PHAs in smoked sausage using sheep casings and cellulose casings were lower during cold storage. It had been proven that PAHs accumulated mainly on the product's surface, due to losses in weight during cold storage and therefore reduced in total PAH.

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تأثير أنواع الأغلفة المختلفة والتخزين على البارد على محتوى الهيدروكربونات العطرية عديدة الحلقات في السجق البقري المدخن

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الملخص

هدفت هذه الدراسة الي تقدير محتوي المركبات الهيدرو كربونية العطرية عديدة الحلاقات في نوعين من السجق البقري المدخن باستخدام طريقة التدخين علي الساخن غير المباشرة مع استخدام نوعين من الأغلفة وهي (الأغلفة الطبيعية والتي تتمثل في أغلفة الاغنام والأغلفة الصناعية والتي تتمثل في أغلفة السليلوز) وتم تخزين المنتجات علي ٤م° لمدة ٩٠ يوم ودراسة تاثير جميع المعاملات السابقة علي محتوي المركبات الهيدرو كربونية .

واظهرت النتائج ان طريقة التدخين علي الساخن غير المباشرة أدت الي ارتفاع نسبة المركبات الهيدرو كربونية عديدة الحلاقات ذات الوزن الجزيئي المنخفض وهي مركبات غير ضارة بينما انخفضت نسبة المركبات الهيدرو كربونية التي لها تاثير مسرطن مثل بنزو (a) بيرين و بنزو(a) أنثراسين وبنزو (b) فلورانثين .

كما اظهرت الدراسة ان اختلاف نوعية الأغلفة له تاثير علي خفض نسبة المركبات الهيدرو كربونية والذي يتوقف علي درجة النفاذية للأغلفة حيث انخفضت نسبة المركبات الهيدرو كربونية في الأغلفة السليلوزية (٥٩٨,٨٦٧١ ملجم / كجم) مقارنة بالأغلفة الطبيعية(٢٦٩١,٠٢٢٩ ملجم / كجم) كما اظهرت الدراسة ان التخزين علي البارد ادي الي خفض نسبة المركبات الهيدروكربونية ذات الوزن الجزيئي المنخفض .

وتأسيسا علي ذلك يمكن التوصية باستخدام طريقة التدخين غير المباشر في المنتجات المدخنة مع تغليف تلك المنتجات بالأغلفة الصناعية قبل عملية التدخين نظرا لانخفاض درجة حرارة التدخين وانخفاض درجة نفاذية الأغلفة مما يؤدي الي حجز تلك المركبات خارج الأغلفة ويمكن التخلص منها بإزالة الغشاء قبل الاستهلاك .