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Effect of Different Smoking Methods on Quality and Safety of Beef Meat

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MEAT products may be contaminated during the smoking process by carcinogenic polycyclic aromatic hydrocarbons (PAHs). Our study investigated the effect of smoking methods using sawdust wood and liquid smoke solution on the presence of PAHs in beef meat. The results showed that smoked beef meat with liquid smoke had moisture content (40.66% wet basis) higher than smoked beef meat with sawdust wood (37.88% wet basis) after the smoking process directly. Total phenolic content decreased from 34.45 to 27.47 mg GAE/100g in smoked beef meat with liquid smoke, while it decreased from 34.26 to 30.89 mg GAE/100g in smoked beef meat with sawdust wood after refrigerated storage at 4°C for 3 weeks. The TBA values ranged between 0.101 to 0.499 and 0.094 to 0.239 mg malonaldehyde/ kg sample in smoked beef meat with liquid smoke and smoked beef meat with sawdust wood, respectively during refrigerated storage at 4°C for 3 weeks. The TVC ranged from $4.2x10^3$ to $3.5 x10^5$ and $6.2 x10^3$ to $9.7 x10^5$ CFU/g in smoked beef meat with liquid smoke and smoked beef meat with sawdust wood, respectively after refrigerated storage at 4°C for 2 weeks. The hydrocarbon compounds were separated by GC-MS and the results showed that the absence of ketones, alkanes, and aldehydes, reduction in aromatic compounds by 33%, and increment in esters by 87.5 % in smoked beef meat with liquid smoke compared to smoked beef meat with sawdust wood. Generally, the smoked beef meat with liquid smoke was more healthy and safe than smoked beef meat with sawdust wood.

Keywords: Beef meat, Smoking methods, Liquid smoke, Polycyclic aromatic hydrocarbons.

Introduction

Meat is an important part of our diet, a major source of protein for humans and contributes valuable nutrients, such as high quality proteins, all essential amino acids, niacin, vitamin B6, vitamin B12, zinc, iron, selenium, phosphorus, endogenous antioxidants and other bioactive substances that support our health (Oz et al., 2010 and Williams, 2007).

Historically, the need to store and transport meat led to the development of different processing techniques, increasing its conservation ability (Foer, 2009). Smoking is one of the oldest technologies for preserving meat products by penetrating meat products with volatiles resulting from wood thermal combustion (Stumpe-Viksna et al., 2008). Commercial smoked meat products are produced more rapidly by liquid smoke in a modern way. Liquid smoke is more environmentally friendly than conventional smoking methods since both wood raw materials for its production and the concentration applied to foods are controlled (Lingbeck et al., 2014).

Meat and meat products, particularly when cooked well, can be a source of exposure to

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chemical carcinogens, such as polycyclic aromatic hydrocarbons (PAHs) and other pyrolysis products that vary with processing and cooking methods, temperature period, and meat type (Le Marchand et al., 2002). The incidence of PAHs in the processed meat is the result of organic matter being combusted during the cooking and smoking processes. The combustion of organic matter during the cooking and smoking processes of meat leads to occur polycyclic aromatic hydrocarbons (PAHs) (Jiménez-Colmenero et al., 2001). PAHs are formed by grilling the meat directly over an open fire and the fat and juices from the meat drip onto the fire, causing flames (Wakabayashi et al., 1995). These flames contain the PAH molecules which adhere to the meat surface. PAHs may also be formed during other processes of food preparation, such as smoking of meats (Cross and Sinha, 2004).

Food safety is a growing concern worldwide and the presence of high levels of polycyclic aromatic hydrocarbons (PAHs) residues as chemical contaminants in food may cause serious threats to the public health (Muyela et al., 2012 and Zelinkova & Wenzl, 2015). PAHs are ubiquitous pollutants of the environment formed primarily during the incomplete combustion of organic materials (e.g. coal, oil, petrol, and wood) (Abdel-Shafy and Mansour**,** 2016). PAHs are known to affect the growth, metabolism and survival of organisms. PAHs are associated with damaging DNA, causing mutations, reproductive toxicity, carcinogenicity and other effects in the organism (ATSDR, 1995, Phillips, 1999 and IARC, 2010).

PAHs are a large group of organic contaminants composed of two or more fused aromatic rings (Sun et al., 2018). Among the hundreds of PAHs formed during the smoking processes, benzo[a] pyrene (BaP) classified as a group 1(carcinogenic to humans) and 16 others assigned either to group 2A (probably carcinogenic) or group 2B (possible carcinogen) are generated (IARC, 2010).

In smoking process, the meat is exposed to the smoke of high-temperature pyrolysed wood chips, which leads to a series of volatile compounds (Aaslyng & Meinert, 2017). According to Flores (2010) the major aroma compounds present in bacon include aldehydes, pyridines, pyrazines, furans, alcohols, and ketones. An alternative to the generation of these compounds from the conventional smoking is the use of condensates from wood smoke, commonly known as liquid smoke. The use of liquid smoke enables greater process control, removes carcinogenic substances, such as polycyclic aromatic hydrocarbons, and gives much of the desired flavor and aroma of traditional smoking (Lingbeck et al., 2014).

The amount of PAHs transmitted by the smoke particles depends on the actual smoking technology, combustion temperature, the smoke composition, type of wood and exposure of the edible parts to the smoke (Duedahl-Olesen et al., 2006). In addition, the amount of PAHs depends on factors such as distance from the heat source, fuel used, processing level, processing periods, and methods, while processes such as reuse, conching, concentration, crushing and storage increase the amount of PAHs in some food products (Singh et al., 2016). According to the European Food Safety Authority, meat and meat products are among the food groups that contribute most to the dietary intake of PAHs per day of European Union member state consumers (EFSA, 2008). This illustrates the important role of PAH studies for smoked food products, in order to measure these compounds and recognize factors that increase PAHs in food.

The present study was aimed to investigate the effect of smoking methods using sawdust wood and liquid smoke solution on the presence of PAHs in beef meat and on the storage period and produce a safe product free from carcinogenic substances of hydrocarbon and has good organoleptic properties and suitable shelf life.

Materials and Methods

Materials

Brazial frozen imported boneless beef meat hindquarter cuts, fresh carrot, tomato, green pepper, onion, garlic and celery, refined fine iodized common salt, spices blend mixture white, red and black pepper, natural smoke concentrate (Meat and Fish Technol. Res. Dept , Agriculture Research Center) and polyethylene bags were obtained from a local market, Alexandria city, Egypt. All reagents and chemicals used in this study were an analytical grade.

Methods

Technological methods

Smoked beef meat preparation: Frozen meat was thawed at room temperature (22 ± 3˚C) for 4-5 hr, dressed by removing their surrounded fat layers, washed and holes in every side of the

meat were made and garlic put in them. The meat was soaked in 15% (w/v) salt solution for 1 hr, then spices were added to meat and left for 1 hr for absorption and distribution in meat, after that meat was cooked in an oven (Modermob-Fresh) for nearly 90 min at 100˚C. The cooked meat was kept in the refrigerator for ~ 8 hr, then cut into small pieces round form with side length ~ 6 cm , after that cooked meat was smoked by two methods: by combustion of sawdust wood and by liquid smoke solution (natural smoke concentrate: water as 1:1, v/v) , since the cooked meat was sprayed by liquid smoke solution each 5 min. The smoking process was carried out in a smoking kiln (AFOS, MK2, Torry mini &maxi smoker, England) at 50˚C for 1hr, 60˚C for 1hr and 70 ˚C for 2 hr, then the samples were obtained and cooled. Finally, they were packaged under vacuum in polyethylene bags and stored at 4˚C for 3 weeks. The smoked meat samples were analyzed at 0, 1, 2, 3 weeks of refrigerated storage.

Analytical methods

Physical properties

The colour values, lightness (L*), redness (a*) and yellowness (b*) of smoked beef meat samples were measured using a Hunter Lab Ultra Scan VIS model, colorimeter (USA). The instrument was standardized during each sample measurement with a black and white tail (L*= 94.1, a*= 1.12, b* =1.26). Five readings of each colour index of Hunter scale (L*, a*, b*) were recorded (Santipanichwing & Suphantharika, 2007).

Texture profile analysis (TPA) of smoked beef meat samples carried out using TA-XT plus Texture Analyzer (Texture Pro CT3 V1.2,Brookfield, Middleboro, USA) as described by Yuan & Chang (2007). Smoked meat sections (height-20 mm) were axially compressed to 40% of their original height. Deformation of force time was obtained with a load cell of 5 kg, applied at a crosshead speed of 1mm/s. Attributes calculated were hardness, cohesiveness, springiness, and chewiness.

Chemical analysis

The moisture content of smoked beef meat samples was determined according to the AOAC (2000)**.** Thiobarbituric acid (TBA) was calorimetrically estimated according to Park et al. (2007) using UV-VIS Spectrophotometer Laxo alpha 1102, suit and expressed as mg malonaldehyde per kilogram fat or sample. The total polyphenol content in the ethanolic (95%) extracts was determined using the Folin-ciocalteu method (Singleton and Rossi, 1965). The concentration of total phenolic compounds was calculated based on the standard curve of gallic acid ($C_6H_2(OH)_3CO_2H$) and the results were expressed as mg gallic acid equivalent (GAE)/100g. The pH was measured at room temperature (22 ± 3°C) using pH meter type MVX100 Beckman as described in AOAC (2000).

Gas chromatographic analysis

Extraction of smoked beef meat samples was carried out according to the procedure described by Mittendorf et al. (2010) with minor modifications. For drying any water present in the sample, the homogenized sample (10 g) was added to a 250-mL E-flask and blended with anhydrous sodium sulfate (15 g). Then, for saponification, 4M methanolic KOH solution (60 mL) was added and the sample was shaken for 25 min in a sealed flask in an ultrasonic bath (Bandelin SONOREX Digital 10P). The saponified sample was filtered into a 250-mL E-flask through glass wool and then hexane (100 mL) was added to the sample and shaken for about 5 min and allowed to stand in order to let the layers separate. The hexane layer was inserted into an E-flask after separation. Methanol: water mixture (4:1 v/v, 50 mL) was used to wash the hexane layer and allowed it to separate, and then the organic phase was collected into the E-flask. The extract was dried with anhydrous sodium sulfate (15 g). The hexane fraction was transferred to a round-bottomed flask and concentrated under reduced pressure at 40˚C to around 2 mL in a rotary evaporator (JAMES. Jobling and Co. Ltd., Staffordshire, UK). Adsorption column chromatography with silica gel (15 g) and anhydrous sodium sulfate (5 g) packed in a glass column (10mm i.d. X 30 cm) was used to clean the concentrated extracts was done. The column was conditioned with hexane (10 mL), then the extract (2 mL) was carefully placed into the column, eluted with hexane (25 mL) and concentrated to 2 mL in a rotary evaporator ready for GC-MS analysis.

Aliquot was analyzed by gas chromatography-mass spectrometry (GC-MS) according to Mahugija & Njale (2018). GC-MS type was Thermo Scientific TRACE 1300 GC (A Trace 1300 GC ultra / Mass spectrophotometer ISQ QD (Thermo Scientific) instrumented x caliber 2.2 software (Thermo x caliber)). For identifying the PAHs, retention times and three relevant ion masses with major spectral abundances were used.

Microbiological methods

Ten grams of smoked beef meat were blended with 90 ml of sterilized peptone water for 5 min in a sterilized glass jar of a blender. Appropriate dilution and the recommended culture media of Oxoid (2002) were prepared for enumeration using standard microbiological pour plate technique. Total viable count (TVC) and Psychrotrophic bacteria count (PC) were performed using plate count agar medium and the plates were incubated at 35-37°C for 48 hrs and 7°C for 10 days, respectively. To detect *Staphylococcus aureus* the recommended Difico Barid Parker agar medium by ICMSF (1978) was used and the plates were incubated at 35-37°C for 48 hrs.

Sensory evaluation

Colour, texture, taste, odour and overall acceptability of smoked beef meat were organoleptically evaluated using 10 trained panelists from, Food Science and Technology Department, Faculty of Agriculture, Alexandria University. They were asked to rate their acceptabilities of smoked beef meat products according to nine-point scale, ranging from the like extreme 9 to dislike extreme 1 point as described by Meilgaard et al. (1999).

Statistical Analysis

Data was statistically analyzed using statistical package for social sciences software (SPSS Statistics V22.0). The level of significant difference was determined at $P \leq 0.05$. Mean ± standard deviation (SD) of mean was used.

Results and Discussion

Moisture content

According to the data in Table 1 smoked beef meat with liquid smoke had moisture content (40.66% wet basis) higher than smoked beef meat with sawdust wood (37.88% wet basis) in the zero time after the smoking process before refrigerated storage. Increment of the moisture content of smoked beef meat with liquid smoke was due to the use of liquid smoke solution. After three weeks of storage at 4°C, moisture content increased by ~ 11% and 14% in smoked beef meat with liquid smoke and smoked beef meat with sawdust wood, respectively. Generally, according to the obtained data and statistical analysis there is a significant difference between the treatments and also between storage periods ($p \leq 0.05$).

Total phenolic content (TPC)

Levels of phenolic compounds were measured and expressed as mg gallic acid equivalent (GAE)/ 100g (Table 1). The results showed that total phenolic content decreased from 34.45 to 27.47 mg GAE/100g in smoked beef meat with liquid smoke, while it decreased from 34.26 to 30.89 mg GAE/100g in smoked beef meat with sawdust wood after refrigerated storage at 4ºC for 3 weeks . The decrement of TPC was 20.26% and 9.84% in smoked beef meat with liquid smoke and smoked beef meat with sawdust wood, respectively. Generally, smoked beef meat with sawdust wood kept TPC more than another product due to the amount of chemical compounds which were observed from smoking with sawdust wood. The results showed that there is no significant difference between the treatments ($p > 0.05$), while there is a significant difference between storage periods ($p \leq 0.05$). Valø *et al.* (2020) showed that TPC content in fish fillets smoked with the atomization of purified condensed smoke was higher with 38% than those smoked with traditional cold smoking by wooden chips.

Colour and texture

Data in Table 2 showed that small differences in colour between smoked beef meat with liquid smoke and smoked beef meat with sawdust wood. Both lightness (L*) and redness (a*) slightly decreased after refrigerated storage at 4ºC for 3 weeks, while yellowness (b*) did not change during storage. In smoked beef meat with liquid smoke, the value of lightness (L*) decreased from 54.83 in zero time to 53.34 after storage, while the value of redness (a*) decreased from 3.11 in zero time to 1.67 after storage. In smoked beef meat with sawdust wood, the value of lightness (L*) decreased from 64.08 in zero time to 60.58 after storage, while the value of redness (a*) decreased from 1.75 in zero time to 0.91 after storage. The values of yellowness (b*) were (16.46, 16.44) in smoked beef meat with liquid smoke, while they were (17.54, 17.23) in smoked beef meat with sawdust wood, in zero time and after storage, respectively. These slight changes are attributed to the increasing of moisture in both smoked beef meat products. Smoked beef meat with liquid smoke was darker and reddish and slightly less yellowish compared to smoked beef meat with sawdust wood.

TABLE 1. Moisture and total phenolic contents of smoked beef meat products after refrigerated storage at 4°C for 3 weeks.

Sample	Moisture content (%, wet basis)		Total Phenolic content (mg GAE*/100g)	
	Storage period (week)		Storage period (week)	
	0	3	0	3
Smoked beef meat with liquid smoke	$40.66\pm0.13^{a}_{b}$	$45.06\pm0.24^{a}_{a}$	$34.45\pm0.65_{a}$	$27.47\pm0.31_{b}$
Smoked beef meat with sawdust wood	$37.88\pm0.65^{b}_{b}$	$43.22\pm0.48^{b}_{a}$	$34.26\pm0.64_{a}$	$30.89\pm0.31_{b}$

*GAE, gallic acid equivalent.
Means in a column not sharing the same superscript letter are significantly different at $p \leq 0.05$, means in a row not sharing the same subscript letter are significantly different at $p \leq 0.05$.

Smoking of sausage with wood chips has lower levels of total cellulose and hemicellulose that generated a lower temperature of smoke production and resulted in a lighter colour of the smoked sausage that may have been attributed to the colour that appeared on the surface of sausages that was associated with cellulose and hemicellulose contents of wood chips. Compounds such as carbonyl groups are necessary substrates for the occurrence of caramelization and Maillard reactions during the pyrolysis of cellulose and hemicellulose, both of which are critical in the presence of colours on the surface of smoked sausage (Toth and Potthast. 1984 and Ledesma et al., 2016). The colouring of meat products in the smoking process is mainly caused by non-enzymatic Maillard browning (Möhler, 1978).

Texture profile analysis (TPA) observed that smoked beef meat with liquid smoke had hardness, chewiness, springiness and gumminess less than smoked beef meat with sawdust wood, as shown in Table 2. On the other hand smoked beef meat with liquid smoke had cohesiveness more than the second product. After refrigerated storage at 4°C for 3 weeks, decreasing in all parameters were happened in both products, except the cohesiveness and springiness which slightly increased in smoked beef meat with liquid smoke, but in smoked beef meat with sawdust wood, cohesiveness slightly decreased, while springiness slightly increased. These changes were attributed to increase the moisture during refrigerated storage and methods of smoking.

Lakshmanan et al. (2005) found that the texture of smoked salmon during refrigerated storage became tough, this increase in muscle firmness due to protein denaturation by high pressure treatment. If meat is thoroughly cooked, particularly with moist heat, collagen fibres may be fully gelatinized, but this may not happen when steaks are lightly cooked. Thermal denaturation of intramuscular collagen usually occurs at 53-63°C (Martens et al., 1982), but denaturation in extramuscular collagenous structures such as ligaments may occur at 70°C (Vangsness et al., 1997). Compounds such as formaldehyde and glyoxal derived from cellulose pyrolysis lead to the occurrence of casing hardening in smoked sausage, since these compounds react with proteins, causing cross-linkage of proteins and then hardening the sausage casing (Toth & Potthast, 1984 and Ledesma et al., 2016).

Rongrong et al. (1998) mentioned that the primary mechanical parameters that can be used to describe the texture properties of smoked sausages are hardness, cohesiveness and springiness. They found that the hardness, cohesiveness and springiness of smoked sausages decreased with increasing water content.

pH and TBA

Slight difference of pH values was observed of smoked beef meat products (Table 3) during refrigerated storage at 4°C for 3 weeks. The pH values ranged from 5.92 to 6.52 in smoked beef meat with liquid smoke, these values were lower than pH values of smoked beef meat with sawdust wood. The pH values of smoked beef meat with sawdust wood ranged from 6.64 to 6.81, since the pH decreased during refrigerated storage for 2 weeks, then it increased after the third week of storage. These changes attributed to microbial activity degrading the meat muscles and increasing pH after 3 weeks due to microbial spoilage that happened. Angsupanich & Ledward (1998) attributed that decrease in cod fish pH after pressurization to denature some protein fractions, while pH increases due to microbial spoilage during refrigerated storage.

The results of TBA values in Table 3 showed that there is a significant difference between the two treatments and also between storage periods ($p \leq 0.05$). The TBA values of smoked beef meat with liquid smoke ranged between 0.101 to 0.499 mg malonaldehyde/ kg sample during refrigerated storage at 4°C for 3 weeks, while the values of TBA in smoked beef meat with sawdust wood ranged between 0.094 to 0.239 mg malonaldehyde/ kg sample. Slightly changes in TBA during storage related to the protective effect from lipid oxidation of antioxidant phenolic derivatives present in smoked beef meat. These values were below the level of incipient rancidity (≥ 1) (Ockerman, 1976). The values of thiobarbituric acid reacting substances remained constant in pressurized and unpressurized smoked dolphinfish cooled at 5°C for 75 days. The phenolic compounds generated from the smoking process prevented lipid oxidation (Gómez-Estaca et al., 2007).

Microbiological quality

The Total Viable Count (TVC), psychrophilic bacteria, and *Staphylococcus aureus* were determined in smoked beef meat products during refrigerated storage at 4°C for 3 weeks. The data in Table 3 showed that TVC ranged from $4.2x10^3$ to $3.5 x10^5$ CFU/g in smoked beef meat with liquid smoke after 2 weeks, while after the third week the spoilage happened and TVC reached to $8.9 x10^6$ CFU/g. The TVC values ranged from $6.2 x10^3$ to $9.7 x10^5$ CFU/g in smoked beef meat with sawdust wood after 2 weeks and the spoilage occurred after the third week when TVC reached to $9.8 x10^6$ CFU/g. There was a small difference between the two smoked beef meat products and the TVC values of smoked beef meat with liquid smoke were less than smoked beef meat with sawdust wood, these related to the presence of organic compounds as a result of the smoking process that have an antibacterial effect, especially ester compounds and these compounds are found in smoked beef meat with liquid smoke by a large amount. Psychrophilic bacteria and *Staphylococcus aureus* were not detected in all samples before and after storage.

Liquid smoke fractions have antimicrobial properties against a variety of Gram-positive and Gram-negative bacteria, yeast, and molds (Milly et al., 2005). Liquid smoke exhibits antimicrobial activity against *Staphylococcus aureus* (Van Loo., 2012). The antimicrobial activity of the liquid smoke may be considered as the existence of phenolic compounds, aldehydes and organic acids that alter the permeability of the membranes of microorganisms, causing membrane damage, leakage of intracellular compounds, especially the gram-positive bacteria (Martin et al., 2010 and Davidson, et al., 2013). Phenolic compounds are formed by lignin pyrolysis; these phenolic compounds play an important role in antimicrobial and antioxidative activity in meat products (Pöhlmann et al., 2013).

TABLE 2. Colour and texture of smoked beef meat products after refrigerated storage at 4°C for 3 Weeks.

Parameter	Smoked beef meat with liquid smoke		Smoked beef meat with sawdust wood	
	Storage period (week)		Storage period (week)	
	0	3	0	3
Colour values				
Lightness (L*)	54.83	53.34	64.08	60.58
Redness (a*)	3.11	1.67	1.75	0.91
Yellowness (b*)	16.46	16.44	17.54	17.23
Texture profile analysis (TPA)				
Hardness (g)	2335	1229	3110	2257
Cohesiveness	0.58	0.70	0.47	0.44
Chewiness (mJ)	36.7	27.9	44.1	30.4
Springiness(mm)	2.76	3.32	3.09	3.13
Gumminess(g)	1355	857	1455	991

TABLE 3. Microbiological quality, pH and TBA of smoked beef meat products during refrigerated storage at 4°C for 3 Weeks.

Property	Type of sample	Storage period (week)			
		0	1	2	3
TVC (CFU/g)	Smoked beef meat with liquid smoke	4.2×10^{3}	1.21×10^{4}	3.5×10^{5}	8.9×10^{6}
	Smoked beef meat with sawdust wood	6.2×10^{3}	5.3×10^{4}	9.7×10^{5}	9.8×10^{6}
pH	Smoked beef meat with liquid smoke	6.52	6.27	6.22	5.92
	Smoked beef meat with sawdust wood	6.77	6.65	6.64	6.81
TBA *mg malonaldhyde/kg sample	Smoked beef meat with liquid smoke	$0.101\pm0.003^{a}_{d}$	$0.203\pm0.000^{a}_{c}$	$0.327\pm0.007^{a}_{b}$	$0.499\pm0.007^{a}_{a}$
	Smoked beef meat with sawdust wood	$0.094\pm0.000^{b}_{d}$	$0.096\pm0.003^{b}_{c}$	$0.172\pm0.007^{b}_{b}$	$0.239\pm0.014^{b}_{a}$

Psychrophilic bacteria and *staphylococcus aureus* were not detected.

⋆Means in a column not sharing the same superscript letter are significantly different at $p \leq 0.05$, means in a row not sharing the same subscript letter are significantly different at $p \leq 0.05$.

Sensory evaluation

The scores for smoked beef meat products given by 10 panelists in Table 4 showed that the smoked beef meat with sawdust wood had slightly higher scores for colour, odour, taste, texture, and overall acceptability than smoked beef meat with liquid smoke. Moreover, all samples were accepted by panelists as very good products, this means that the smoking process keeps the quality of beef meat for 2 weeks under refrigeration condition. Low levels of microbial contamination and rancidity in smoked samples lead to the absence of spoiled-related odour. Generally, according to the obtained data and statistical analysis, there is no significant difference between the treatments and also between storage periods ($p > 0.05$).

Hydrocarbon compounds

Table 5 summarizes the chemical components separated by GC-MS for sawdust wood smoke, liquid smoke solution, smoked beef meat with sawdust wood, and smoked beef meat with liquid smoke. The main components of smoked samples were organized into 7 groups: acids, alcohols, aldehydes, alkanes, aromatic compounds, esters, and ketones.

Sawdust wood smoke had 29 compounds were composed of acid, aldehyde, 11 alkanes, 8 aromatic compounds, 4 esters, and 4 ketones, while liquid smoke solution had 19 compounds were composed of acid, alcohol, aldehyde, 5 alkanes, 3 aromatic compounds, 6 esters, and 2 ketones. Moreover smoked beef meat with sawdust wood had 19 compounds were composed of 2 acids, aldehyde, 4 alkanes, 3 aromatic compounds, 8 esters, and ketone, while smoked beef meat with liquid smoke had 19 compounds were composed of 2 acids, 2 aromatic compounds, and 15 esters .

Tables 6 - 9 show the major and minor compounds of sawdust wood smoke, liquid smoke solution, smoked beef meat with sawdust wood and smoked beef meat with liquid smoke. They showed the chemical compound, its chemical group, area (%), molecular formula, and molecular weight. From these results we noticed that: The sawdust wood smoke contained higher number of chemical compounds compared to other samples. Also, it contained a higher number of aromatic compounds, ketones, and alkane and a lower number of esters than the liquid smoke solution.

According to Woods (2003) wood smoke consists of more than 400 volatile components, containing 48 acids, 22 alcohols, 131 carbonyls,

22 esters, 46 furans, 16 lactones, 75 phenols, and 50 miscellaneous compounds. Möhler (1978) and Lingbeck et al., 2014 reported that in the smoking process, compounds have undesirable effects on products such as phenol, acetone, and unsaturated long-chain compounds (undesirable aroma). PAHs are the most important among undesirable compounds. The most significant endpoint of toxicity with PAHs is cancer.

Chrysene is a PAH compound, it was found in sawdust wood smoke (Table 6) and its area was 1.61%, but it was not found in liquid smoke solution (Table 7), this compound has a health hazard. Esters were found in sawdust wood smoke with area 20.15%, while they were found in liquid smoke solution with area 42.45%. Octadecenoic acid was found in liquid smoke solution with area 2.31 %, but it was not found in sawdust wood smoke. This means that the liquid smoke solution is more healthy than sawdust wood smoke because esters and acid have antibacterial effects and safe for health. Lingbeck et al., 2014 mentioned that liquid smoke is generated by condensing wood smoke formed by regulated pyrolysis of sawdust or wood chips with minimal oxygen content. Then it refined and filtered to remove toxic and carcinogenic impurities containing PAHs. The liquid is eventually aged for mellowness.

Tetradecanoic acid and Hexadecanoic acid have the property of antioxidant and antimicrobial activities. 9, 12, Octadecadienoic acid (Z,Z) – has the property of anti-inflammatory and antiarthritic as reported by Lalitharani et al. (2010). Although PAHs are extremely toxic, they have low water solubility which allows liquid smoke manufacturers to easily separate out these compounds from their finished products using phase separation and filtration techniques (Guillén and Sopelana, 2003). PAH levels in foods have been regulated through EC Regulation No 208/2005 (EC, 2005) and Commission Regulation No 1881/2006 of the European Union (EU) (EU, 2011). Benzo[a]pyrene (BaP) was set as the marker for the occurrence and effect of carcinogenic PAHs in food, also PAHs include benz[a] anthracene, benzo[b] fluoranthene, benzo[j] fluoranthene, benzo[k] fluoranthene, benzo[ghi] perylene, chrysene, cyclopenta[cd] pyrene, dibenz[a,h] anthracene, dibenzo[a,e] pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene. The maximum level of 5.0 mg/kg BaP was set for smoked meats and smoked meat products in this EC regulation (WHO, 2006).

Esters were found as major compounds with area 75.56% in smoked beef meat with sawdust wood (Table 8), since the number of esters reached to 8 compounds, followed by alkanes (4 compounds). A high reduction in aromatic compounds was noticed from 8 compounds in sawdust wood smoke to 3 compounds in smoked beef meat with sawdust wood and chrysene was not found in smoked beef meat with sawdust wood smoke. Moreover, Hexadecanoic acid methyl ester was found with an area of 30.93%. In sawdust wood smoke scytlone was a major compound with area11.18% (Table 6), while it was the absence of smoked beef meat with sawdust wood smoke. Volatile compounds resulting from the thermal combustion of wood penetrated meat products about 0.5 cm from the surface, so these compounds in meat products were less than in sawdust wood smoke.

TABLE 4. Sensory evaluation of smoked beef meat products during refrigerated storage at 4°C for 2 weeks.

Property	Smoked beef meat with liquid smoke			Smoked beef meat with sawdust wood		
	Storage period (week)			Storage period (week)		
	0	1	2	0	1	2
Colour	7.73	7.73	7.20	8.09	7.55	7.40
Odour	7.18	7.45	7.70	8.18	7.45	8.10
Taste	7.36	7.00	7.20	8.14	7.55	7.40
Texture	7.18	7.18	7.50	8.41	7.91	7.70
Overall acceptability	7.18	7.05	7.65	8.27	7.77	7.50

TABLE 5. Summarized results of hydrocarbons compounds separated by GC –MS.

Products	Hydrocarbons compounds	Chemical group							
		Acids	Alcohol	Aldehydes	Alkanes	Aromatic compounds	Esters	Ketones	Total
Sawdust wood smoke	Number	1	0	1	11	8	4	4	29
	Range (%)	(2.25)	-	(0.54)	(1.22 - 7.01)	(1.06 - 4.21)	(0.65 - 10.08)	(0.88 - 11.18)	
	Total area (%)	2.25	-	0.54	32.24	16.03	20.15	20.23	
Liquid smoke solution	Number	1	1	1	5	3	6	2	19
	Range (%)	2.31	2.16	0.48	(1.39 - 10.57)	(1.11- 4.23)	(0.57 - 19.51)	(0.45 - 2.78)	
	Total area (%)	2.31	2.16	0.48	24.5	6.55	42.45	3.23	
Smoked beef meat with sawdust wood	Number	2	0	1	4	3	8	1	19
	Range (%)	(0.87 - 2.35)	-	1.15	(0.24-1.03)	(0.39 - 2.22)	(0.30 - 30.93)	0.87	
	Total area (%)	3.22	-	1.15	1.85	2.94	75.56	0.87	
Smoked beef meat with liquid smoke	Number	2	0	0	0	2	15	0	19
	Range (%)	(0.47 - 1.42)	-	-	-	(0.30 - 0.40)	(0.28 - 27.28)	-	
	Total area (%)	1.89	-	-	-	0.70	92.84	-	

TABLE 6. Major and minor chemical compounds of sawdust wood smoke.

Chemical Compound	Area %	Molecular Formula	Molecular Weight
Chemical group:			
Acids:-			
5,8,11,14-Eicosatetraynoic acid	2.25	C20H24O2	296
Aldehyde:			
4-Octadecenal	0.54	C18H34O	266
Alkanes:			
Octacosane	7.01	C28H58	394
Heptadecane	5.14	C17H36	240
Heneicosane	4.32	C21H44	296
Octadecane,2,6-dimethyl	3.36	C20H42	282
Eicosane,10-methyl	3.26	C21H44	296
Tetratetracontane	2.70	C44H90	618
Octadecane,5-methyl	1.70	C19H40	268
Dodecane,5,8-diethyl-	1.54	C16H34	226
Heptadecane,9-hexyl	1.41	C23H48	324
Heptacosane	1.22	C27H56	380
Octadecane,3-ethyl5-(2-ethylbutyl	0.58	C26H54	366
Aromatic compounds:			
1-Phenanthrenecarboxylic acid,1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-,methyl ester,[1R-(1à,4aá,10aà)]-	4.21	C21H30O2	314
1,3-Benzodioxole,5,5'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-,[1S-(1à,3aà,4á,6aà)]-	2.77	C20H18O6	354
Cyclopropa[3,4]cyclohepta[1,2-a] naphthalene,1,1a,1b,2 , 3,7b,8,9,10,10adecahydro-5-methoxy-10-methylene	2.60	C18H22O	254
Chrysene,1,2,3,4,4a,4b,5,6,10,10a,10b,11-dodecahydro	1.61	C18H24	240
10,18-Bisnorabieta-5,7,9(10),11,13-pentaene	1.50	C18H22	238
1-Phenanthrenecarb oxylic acid,7-ethenyl1,2,3,4,4a,4b,5,6, 7,9,10,10a-dodecahydro1,4a,7-trimethyl-,methyl ester,[1R-(1à,4aá,4bà,7à,10aà)]-	1.16	C21H32O2	316
4b,8-Dimethyl-2-isopropylphenanthrene,4b,5,6,7, 8,8a , 9,10-octahydro	1.12	C19H28	256
1-Phenanthrenecarboxylic acid,1,2,3,4,4a,10a-hexahydro -1,4a-dimethyl-7-(1-methylethyl)-,methyl ester,[1R- (1à,4aá,10aà)]-	1.06	C21H28O2	312
Esters:			
Pentadecanoic acid, 14-methyl-,methyl ester	10.08	C17H34O2	270
Heptadecanoicacid, 16-methyl-,methyl ester	4.81	C19H38O2	298
6-Octadecenoic acid, methyl ester,(Z)-	4.61	C19H36O2	296
Methyl tetradecanoate	0.65	C15H30O2	242
Ketones:			
Scytalone	11.18	C10H10O4	194
1-(10-Methylanthracen-9-yl)ethanone	6.82	C17H14O	234
1,8-Dioxacyclohexadecane-2,10-dione,5,6:12,13-diepoxy 8,16-dimethyl-	1.35	C16H24O6	312
2,2',4,4'-Tetramethyl diphenylsulphone	0.88	C16H18O2S	274

TABLE 7. Major and minor chemical compounds of Egyptian commercial liquid smoke solution.

Chemical Compound	Area %	Molecular Formula	Molecular Weight
Chemical group:			
Acids:			
6-Octadecenoic acid, (Z)-	2.31	C18H34O2	282
Alcohol:			
1-Heptatriacotanol	2.16	C37H76O	536
Aldehyde:			
Nonanal	0.48	C9H18O	142
Alkanes:			
2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo (3.3.0)octane	10.57	C20H18O6	354
Octadecane,5,14-dibutyl	6.92	C26H54	366
Stigmastan-6,22-dien, 3,5-dedihydro	2.99	C29H46	394
Tetratetracontane	2.63	C44H90	618
Octadecane,3-ethyl-5-(2-ethylbutyl)-	1.39	C26H54	366
Aromatic compounds:			
Card-20(22)-enolide,3,5,14,19-tetrahydroxy-,(3á,5á)-	4.23	C23H34O6	406
1,3-Benzodioxole,5,5-'(tetrahydro-1H,3H-furo[3,4-c]furan 1,4-diyl)bis-,[1S-(1à,3aà,4á,6aà)]-	1.21	C20H18O6	354
Estragole	1.11	C10H12O	148
Esters:			
Phthalic acid, butyl 2-pentyl ester	19.51	C17H24O4	292
Ergost-5-en-3-ol,acetate, (3á,24R	13.25	C30H50O2	442
Hexadecanoic acid, methyl ester	5.06	C17H34O2	270
10-Octadecenoic acid, methyl ester	2.62	C19H36O2	296
Heptadecanoic acid, 16-methyl-,methyl ester	1.44	C19H38O2	298
1,2-Benzenedicarb oxylic acid,diisooctyl ester	0.57	C24H38O4	390
Ketones:			
9,10-Secocholesta5,7,10(19)-triene-3,25,26-triol,(3á,5Z,7E)-	2.78	C27H44O3	416
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9 diene-2,8-dione	0.45	C17H24O3	276

TABLE 8. Major and minor chemical compounds of smoked beef meat with sawdust wood.

Chemical Compound	Area %	Molecular Formula	Molecular Weight
Chemical group:			
Acids:-			
Pentadecanoic acid	2.35	C15H30O2	242
Oxiraneoctanoic acid,3-octyl-,cis	0.87	C18H34O3	298
Aldehyde:			
17-Octadecenal	1.15	C18H34O	266
Alkanes:			
Heneicosane	1.03	C21H44	296
Trilostane	0.30	C20H27NO3	329
Octadecane,3-ethyl-5-(2ethylbutyl)	0.28	C26H54	366
Heptadecane, 9-hexyl-	0.24	C23H48	324
Aromatic compounds:			
1,2-Benzenedicarboxylic acid, diisooctyl ester	2.22	C24H38O4	390
4-HCyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]Oxiren-4-one,8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8a-trihydroxy2a(hydroxyl methyl) 1,1,5,7-tetramethyl-, (1aà,1bá,1cá,2aá,3aá,6aà,6bà, 7à,8á,8aà)-	0.39	C22H30O8	422
Bicyclo[2.2.1]heptane,2,2,3,5,5-pentachloro-7,7-bis(chloromethyl)-1-dichoromethyl	0.33	C10H9Cl9	444
Esters:			
Hexadecanoic acid, methyl ester	30.93	C17H34O2	270
Octadecanoic acid, methyl ester	23.40	C19H38O2	298
10-Octadecenoic acid, methyl ester	17.06	C19H36O2	296
Hexadecanoic acid, ethyl ester	1.29	C18H36O2	284
Octadecanoic acid, ethyl ester	0.97	C20H40O2	312
7-Hexadecenoic acid, methyl ester Z	0.90	C17H32O2	268
Pentadecanoicacid,14-methyl,methyl ester	0.71	C17H34O2	270
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-	0.30	C28H44O4	444
Ketone:			
Androst-5-en3-one,19-acetoxy-4,4-dimethyl,oxime	0.87	C23H35NO3	373

TABLE 9. Major and minor chemical compounds of smoked beef meat with liquid smoke.

Chemical Compound	Area %	Molecular Formula	Molecular Weight
Chemical group:			
Acids:			
Octadecanoic acid	1.42	C18H36O2	284
8,11,14-Eicosatrienoic acid,(Z,Z,Z)-	0.47	C20H34O2	306
Aromatic compounds:			
2,6,10,14,18,22-Tetracosahexaene,2,6,10,15 ,19,23-hexamethyl-, (allE)-	0.40	C30H50	410
1,2-Benzenedicarboxylic acid, diisooctyl ester	0.30	C24H38O4	390
Esters:			
Hexadecanoic acid, methyl ester	27.28	C17H34O2	270
Octadecanoic acid, methyl ester	26.29	C19H38O2	298
9-Octadecenoic acid (Z)-,methyl ester	18.12	C19H36O2	296
Methyl tetradecanoate	5.20	C15H30O2	242
9-Hexadecenoic acid, methyl ester, (Z)-	4.44	C17H32O2	268
(E)-9-Octadecenoic acid ethyl ester	2.99	C20H38O2	310
Hexadecanoic acid, ethyl ester	1.58	C18H36O2	284
Octadecanoic acid	1.42	C18H36O2	284
Methyl Z-11-tetradecenoate	1.16	C15H28O2	240
Cyclopropaneoctanoicacid, 2-hexyl,methyl ester	1.07	C18H34O2	282
Octadecanoic acid, ethyl ester	1.05	C20H40O2	312
Tetradecanoic acid,12-methyl-,methyl ester	0.89	C16H32O2	256
Hexadecanoic acid,14-methyl-, methyl ester	0.70	C18H36O2	284
Tridecanoic acid, methyl ester	0.37	C14H28O2	228
Hexadecanoic acid, ethyl ester	0.28	C18H36O2	284

Comparing the compounds in liquid smoke solution (Table 7) to smoked beef meat with liquid smoke (Table 9) showed that absence of aldehydes, ketones and alkanes in smoked beef meat with liquid smoke. The reduction in the number of aromatic compounds was 33.3%, while increment in the number of esters reached to 150% in smoked beef meat with liquid smoke. Hexadecanoic acid methyl ester was found as a major compound with 27.28 % area, followed by octadecanoic acid methyl ester with area 26.29 % area in smoked beef meat with liquid smoke. While phthalic acid, butyl 2-pentyl ester was found as a major compound with area 19.51% in liquid smoke solution. Liquid smoke is easier to apply than conventional smoking and allows the desired characteristics to be reproducible in the end product (Lingbeck et al., 2014).

The results (Tables 5, 8 & 9) showed that the absence of ketones, alkanes, and aldehydes, reduction in aromatic compounds by 33%, and increment in esters by 87.5 % in smoked beef meat with liquid smoke compared to smoked beef meat with sawdust wood. From the above results smoked beef meat with liquid smoke was more healthy and safe than smoked beef meat with sawdust wood.

Conclusion

The use of the liquid smoke solution in the smoking process of beef meat produced a healthy product had two weeks shelf life at 4°C due to antioxidant and antibacterial compounds which were found in the product as a result of smoking process. The product was acceptable in organoleptic properties and had good colour and texture, also it contained a considerable amount of compound which has antimicrobial activity and it was free from carcinogenic compounds (PAHs) as chrysene that was found in sawdust wood smoke. The use of liquid smoke in the food industry allows higher process control, eliminates carcinogenic compounds, such as PAHs, and this may satisfy consumer demand for all natural foods, also it imparts a pleasant flavor and also has an inhibitory effect on pathogenic bacteria.

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تأثير طرق التدخين المختلفة على جودة وسلامة اللحم البقري

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نظراً لأمكانية حدوث تلوث في منتجات اللحوم بالهيدروكربونات العطرية متعددة الحلقات المسببة للسرطان (PAHs) أثناء عملية التدخين. تم إجراء هذا البحث لدراسة تأثير طرق التدخين باستخدام نشارة الخشب ومحلول سائل التدخين على وجود الهيدروكربونات العطرية متعددة الحلقات في اللحم البقري. أظهرت النتائج أن محتوى الرطوبة في اللحم البقري المدخن بسائل التدخين (٤٠,٦٦ على أساس وزن رطب) أعلى من اللحم البقري المدخن بنشارة الخشب (٣٧,٨٨٪ على أساس وزن رطب) بعد عملية التدخين مباشرة . كما انخفض محتوى الفينولات الكلية من ٣٤,٤٥ إلى ٢٧,٤٧ مجم GAE /١٠٠جم في اللحم البقري المدخن بسائل التدخين ، بينما انخفض من ٣٤,٢٦ إلى ٣٠,٨٩ مجم GAE /١٠٠جم في اللحم البقري المدخن بنشارة الخشب بعد التخزين المبرد على ٤°م لمدة ٣ أسابيع. أيضاً تراوحت قيم حمض الثيوباربيوتريك أثناء التخزين المبرد على ٤°م لمدة ٣ أسابيع بين ٠,١٠١ إلى ٠,٤٩٩ ,و ٠,٠٩٤إلى ٠,٢٣٩ مجم مالون ألدهيد/ كجم عينة في اللحم البقري المدخن بسائل التدخين واللحم البقري المدخن بنشارة الخشب على الرتيب. وأظهرت نتائج التحاليل الميكروبيولوجية ان العد الكلي المتاح بعد التخزين المبرد عند ٤°م لمدة أسبوعين تراوح من $4.2x10^3$ إلى $3.5x10^5$ و $6.2x10^3$ إلى $9.7x10^5$ CFU/g في اللحم البقري المدخن بسائل التدخين واللحم البقري المدخن بنشارة الخشب على الترتيب. وقد تم فصل المركبات الهيدروكربونية بواسطة GC–MS وأظهرت النتائج عدم وجود الكيتونات والألكانات والألدهيدات وانخفاض المركبات العطرية بنسبة ٣٣٪ وزيادة الإسترات بنسبة ٨٧,٥٪ في اللحم البقري المدخن بسائل التدخين مقارنة باللحم البقري المدخن بنشارة الخشب. بشكل عام , فإن اللحم البقري المدخن بسائل التدخين أكثر صحية وأمانًا من اللحم البقري المدخن بنشارة الخشب.