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Influence of wood types on quality and carcinogenic polycyclic aromatic hydrocarbons (PAHs) of smoked sausages

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ABSTRACT

Our study investigated the effect of smoking sausages using different types of woodchips—Neem (*Azadirachta indica* Valeton.), Copper pod (*Cassia siamea* Lamk), Earleaf acacia (*Acacia auriculiformis* Cunn.), and Eucalyptus (*Eucalyptus camaldulensis* Dehn)—compared to commercial Beech woodchips on different qualities of the smoked sausages—pH, water acidity, total acidity, color, sensory properties and shelf life. Moreover, the contents of carcinogenic polycyclic aromatic hydrocarbons (PAHs) of the smoked sausages were evaluated using gas chromatography-mass spectrometry (GC-MS). The results indicated that sausage smoked with Neem woodchips had similar physical qualities, sensory properties and shelf-life (9 weeks) to sausage smoked with Beech woodchips. Total PAHs revealed no significant differences between these two sausages, while the PAH4 levels of sausage smoked with Neem woodchips (1.06 μg/kg) was significantly lower than that of sausage smoked with Beech woodchips (1.54 μg/kg). The sausages smoked with Copper pod woodchips (L^* value 54.1, hardness 13.0 N) and Eucalyptus woodchips (L^* value 50.3, hardness 11.1 N) had lighter color and softer texture than those smoked with Beech woodchips (L^* value 50.8, hardness 13.7 N), respectively, while their total PAH and PAH4 contents were not significantly different. Although the sausage smoked with Earleaf acacia woodchips had the lowest contents of total PAHs, its quality during the 9 weeks of storage was unacceptable due to higher aerobic plate counts which were believed to be related to the low smoke generation temperature of the Earleaf acacia woodchips. Therefore, the overall results suggested that the Neem woodchips could be potentially utilized as a low cost alternative to commercial Beech woodchips for smoking meat products in the meat industry.

1. Introduction

Smoking is one of the oldest technologies for conserving meat products with the process involving the penetration of meat products by volatiles resulting from the thermal combustion of wood (Stumpe-Vīksna, Bartkevičs, Kukāre, & Morozovs, 2008). Smoke is formed due to the partial burning of wood during the smoking process. In general, wood consists of approximately 50% cellulose, 25% hemicellulose and 25% lignin (Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2012). The thermal degradation of hemicellulose, cellulose and lignin proceeds at 180–300 °C, 260–350 °C and 300–500 °C, respectively (Šimko, 2011). Smoking produces unique organoleptic properties in and subsequent shelf-life extension of smoked sausages. The pyrolysis of cellulose and hemicelluloses forms significant amounts of carbonyl compounds, which produce the brown color on the surface of smoked meats, while the pyrolysis of lignin forms some phenolic compounds which give the desirable flavor and act as antimicrobial and antioxidative compounds for smoked sausage (Kjällstrand & Petersson, 2001). Furthermore, smoking extends the sausage shelf-life due to the formation of various compounds which contain antimicrobials and antioxidants (Ledesma, Rendueles, & Díaz, 2016). However, as an undesired consequence of smoking, the incomplete combustion and pyrolysis of wood leads to the possibility of generating cancer-producing polycyclic aromatic hydrocarbons (PAHs) (Hitzel, Pöhlmann, Schwägele, Speer, & Jira, 2013).

PAHs are composed of the largest group of chemical agents and have been demonstrated to be carcinogenic and mutagenic (Wenzl, Simon, Anklam, & Kleiner, 2006). Heavy PAHs contain five or more fused aromatic rings and have higher stability and toxicity than light PAHs which contain two to four aromatic rings in their chemical structure (Plaza-Bolaños, Frenich, & Vidal, 2010). Benzo[*a*]

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pyrene (B*a*P) is carcinogenic to humans (group 1), whereas benz[*a*]anthracene (B*a*A), chrysene (Chry), benzo[*k*]fluoranthene (B*k*F) and benzo[*b*]fluoranthene (B*b*F) are possibly carcinogenic to humans (group 2B) (IARC, 2010). PAH 4 (B*a*P, Chry, B*a*A and B*b*F) and PAH16 [naphthalene (Nap), acenaphthylene (Anl), acenaphthene (Ane), fluorene (Flu), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), B*a*A, Chry, B*b*F, benzo[*k*]fluoranthene (B*k*F), B*a*P, indeno[*1,2,3-cd*]pyrene (I*n*P), dibenz[*a,h*]anthracene (D*ah*A), and benzo[*ghi*]perylene (B*ghi*P)] have been commonly used as markers for the occurrence of PAHs in food (Bojes & Pope, 2007; EFSA, 2008). Scientific evidence has indicated a strong association between the consumption of processed meat and increased cancer risk, especially colorectal cancer (Oostindjer et al., 2014). Recently, the International Agency for Research on Cancer (IARC) categorized processed meat and red meat as carcinogenic and probably carcinogenic to humans (groups 1 and 2A), respectively, based on epidemiological studies reporting a correlation with cancer (Bouvard et al., 2015). Therefore, efforts to understand and reduce PAH formation are important and deserve continuing encouragement.

The PAH contents of smoked foods depend on parameters such as the moisture content of the wood used for smoking, the temperature the wood attains during combustion, the concentration of oxygen and the ventilator velocity in the combustion chamber (Hitzel et al., 2013; Škaljac et al., 2014). The type of wood plays a significant role in the contamination of the PAH contents in smoked meat products. Meat smoked with softwood was reported to have higher PAH levels than that of hardwood probably resulting from the chemical compounds in the wood and the different natures of the wood types (Stumpe-Vīksna et al., 2008). Beech (*Fagus sylvatica*) woodchips are an ideal, commercial heat source for smoking meat products globally as they produce good quality smoke and are responsible for providing smoked meat products with highly acceptable color, aroma and flavor (Hitzel et al., 2013). An increased demand for smoked meat products throughout the world has presented a major challenge for meat manufacturers especially in Asia where they must overcome several difficulties associated with importing Beech woodchips from European countries, especially the cost, transportation duration and the uncertain supply (Rotkanok, Angskun, & Angskun, 2013). Moreover, recent studies revealed that European Beech forests are in decline due to extreme drought, heat waves and heavy rain from climate change which are affecting their regenerative growth and mortality, and more broadly the growth and mortality throughout the entire range of growth conditions for the species (Kirchen et al., 2017). Thus, alternative species of woodchips that can provide equal or better quality and safety for smoked meat products would be advantageous to the global meat industry.

Our study investigated the potential of four species of woodchips from fast-growing hardwoods cultivated in Asia—namely Neem (*Azadirachta indica* Valeton.), Copper pod (*Cassia siamea* Lamk), Earleaf acacia (*Acacia auriculiformis* Cunn.) and Eucalyptus (*Eucalyptus camaldulensis* Dehn) by comparison to Beech woodchips, regarding their contents of carcinogenic PAHs when used to smoke sausages. Moreover, the qualities of the smoked sausage (pH, water activity, total acidity, color, sensory properties and shelf life) during storage were also studied. These four-wood species are currently underutilized due to a lack of information and awareness of their potential value. Therefore, this was the first study to introduce the use of these woods for meat smoking with consideration of the quality and safety of the product and comparison to commercial beech woodchips.

2. Materials and methods

2.1. Chemicals

All solvents used in this study were obtained from RCI Labscan (Bangkok, Thailand) and were of: analytical (AR) grade—hydrochloric acid (HCl), sulfuric acid (H_2SO_4), methanol, 2-propanol, 1-buthanol and *n*-hexane—or high-performance liquid chromatography (HPLC) grade—acetonitrile and dichloromethane. EPA 16 PAH standards (part number: 4S8905) consisted of naphthalene (Nap), acenaphthylene (Anl), acenaphthene (Ane), fluorene (Flu), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (B*a*A), chrysene (Chry), benzo[*b*]fluoranthene (B*b*F), benzo[*k*]fluoranthene (B*k*F), benzo[*a*]pyrene (B*a*P), indeno[*1,2,3-cd*]pyrene (I*n*P), dibenz [*a,h*]anthracene (D*ah*A) and benzo[*ghi*]perylene (B*ghi*P). These standards were purchased from Supelco (PA, USA). Internal standards (Acenaphthene-d_{10} and Chrysene-d_{12}) were purchased from Dr. Ehrenstorfer (Augsburg, Germany), potassium hydroxide from Ajax Finechem (NSW, Australia), the solid-phase extraction cartridge (SPE, 6 mL/1000 mg) from Macherey-Nagel, (Düren, Germany), 0.45 μm nylon filters, 2-thiobarbituric acid (TBA) and 1,1,3,3-tetraethoxypropane (TEP) from Sigma-Aldrich (MO, USA).

2.2. Preparation of woodchips

Commercial beech woodchips were obtained from "Räuchergold®" smoking chips (Rosenberg, Germany). Neem, Copper pod, Earleaf acacia, and Eucalyptus woods were obtained from the Sakaerat Environmental Research Station Sakaerat Biosphere Reserve, Wang Nam Khieo, Nakhon Ratchasima, Thailand. These woods were dried at 70 °C for 24 h, cut into small pieces and converted into woodchips. All woodchips were 6.6–7.9 mm in size, and the moisture of the woodchips ranged from 8.3 to 10.2% before being submitted to the following combustion procedures.

2.3. Analysis of moisture content woodchips

The moisture content of woodchips in this research was measured by the gravimetric method following European standard UNI EN 14774-2 (UNI EN, 2010). The sample with the minimum mass of 300 g was dried at 105 ± 2 °C until constant mass was obtained and moisture percentage was calculated from the loss in sample mass using the following equation:

$$\%\text{Moisture} = \frac{(\text{weight of woodchips before drying} - \text{weight of woodchips after drying})}{\text{weight of woodchips before drying}} \times 100 \quad (1)$$

2.4. Analysis of chemical compounds in woodchips

Fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin