



Vegetable oil as fat replacer inhibits formation of heterocyclic amines and polycyclic aromatic hydrocarbons in reduced fat pork patties



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2-Amino-3,4-dimethylimidazo[4,5-f]

quinoline (PubChem CID: 62274)

2-Amino-3,8-dimethylimidazo[4,5-f]

quinoxaline (PubChem CID: 62275)

2-Amino-3,4,8-trimethylimidazo[4,5-f]

quinoxaline (PubChem CID: 104739)

2-Amino-1-methyl-6-phenylimidazo[4,5-b]

pyridine (PubChem CID: 1530)

Benzo[a]pyrene (PubChem CID: 2336)

Benz[a]anthracene (PubChem CID: 5954)

ABSTRACT

Formation of heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) was examined to evaluate the impact of using vegetable oil as fat replacement on carcinogen formation in meat product. Pork patties were formulated with 40% fat replacement by olive oil, sunflower oil or grape seed oil, respectively and cooked at 180 °C or 220 °C. Control patties contained the highest amount of HCAs compared with all other patties at both temperatures. Olive oil and sunflower oil replacement completely inhibited formation of MeIQ (2-amino-3, 4-dimethylimidazo[4,5-f]quinoline), while grape seed oil completely inhibited MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline), 4,8-DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) in patties. Grape seed oil achieved the highest inhibition capacity compared with sunflower oil and olive oil. HCAs increased significantly with cooking temperature ($p < 0.05$), but no difference was observed in total PAHs for patties cooked at different temperature ($p > 0.05$). In conclusion, fat replacement with sunflower oil, olive oil or grape seed oil in pork patties could reduce the formation of HCAs without compromising eating quality.

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1. Introduction

Fat plays an important role in the human diet. It not only creates a unique sensation of food, but also helps maintain health. The consumption of pork in the world has dramatically increased from 18 to 110 million tons per year (1950–2010) (Brown, 2013). Research found that increased saturated fatty acids intake could elevate the risk of cardiovascular disease, but monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) could reduce the risk and maintain cardiovascular health (McAfee et al., 2010; Sadler, 2014). Therefore, changing fatty acids profile of meat products by replacing saturated fatty acids with unsaturated

fatty acids has attracted lots of attention in both academic research and meat processors. Adding olive oil could dramatically increase the percentage of MUFAs in final products, whereas sunflower oil and grape seed oil could greatly raise the level of PUFAs in fat replaced meat products (Gunstone, 2002; Matthäus, 2008). Rodríguez-Carpena, Morcuende, and Estévez (2012) successfully replaced 50% fat with avocado, sunflower and olive oil in cooked pork patties and reported that avocado and olive oil could even offer better aroma to the final products than control ones. Vural and Javidipour (2002) successfully substituted beef fat in Frankfurters with the mixture of interesterified palm, cottonseed and olive oil without changing physical parameters and total sensory scores. Choi et al. (2010) used pre-emulsified grape seed oil and 2% rice bran fibre to develop pork batters with 50% fat replacement and reported that the fat-reduced pork batters could achieve the

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comparable eating quality with control samples. Domínguez, Agregán, Gonçalves, and Lorenzo (2016) replaced 100% pork back fat with olive oil in pork pâté, which significantly increase the content of tocopherol and MUFAs in cooked products without altering physio-chemical properties. Domínguez, Pateiro, Agregán, and Lorenzo (2017) and Lorenzo, Munekata, Pateiro, Campagnol, and Domínguez (2016) replaced 25%–75% back fat with olive oil, microencapsulated fish oil and the mixture of fish oil and olive oil, which significantly increased the percentage of PUFAs in frankfurter type sausage and Spanish *salchichón*. These results indicate that vegetable oils could be used successfully to replace fat partially or completely to offer products comparable eating quality with healthier fatty acids profile, i.e. high level of MUFAs and PUFAs.

However, unsaturated fatty acids in vegetable oils may pose risk in domestic cooking due to their oxidation and decomposition at high temperature. For example, linoleic acid was found associated with the formation of potentially toxic compounds, such as free radicals, aldehydes and ketones (Guillén & Uriarte, 2012a; Katragadda, Fullana, Sidhu, & Carbonell-Barrachina, 2010). These reactive oxygen species (ROS) initiated by unsaturated fatty acids peroxidation could induce the decomposition of Amadori compounds and generate 1- and 3- deoxysone that are intermediates for Strecker aldehydes, pyrazines and pyridines in Maillard reaction. Consequently, it might promote the formation of heterocyclic amines (HCAs) (Morello, Shahidi, & Ho, 2002; Turesky, 2010; Zamora & Hidalgo, 2007). Effect of fatty acids/oils on the formation of HCAs has been documented in previous research. Johansson, Fredholm, Bjerne, and Jägerstad (1995) reported that the higher level of MeIQx and DiMeIQx were found in burgers fried in rapeseed oil containing high level of oleic acid with high peroxides values, compared with butter, margarine and sunflower oil. Zamora, Alcón, and Hidalgo (2012) stated that both primary and secondary lipid oxidation products, hydroperoxides, such as methyl 13-hydroperoxyoctadeca-9,11-dienoate and alkenals could accelerate the formation of PhIP in chemical model system. Some hydroperoxides generated from the decomposition of the unsaturated hydrocarbons during heating, such as linolenate could also undertake aromatization and de-hydrocyclization, further cleave into benzaldehydes and other benzene ring-containing compounds, which are precursors of polycyclic aromatic hydrocarbons (PAHs) (Chen & Chen, 2001; Lorenzo, Purriños, Fontán, & Franco, 2010; Lorenzo et al., 2011; Singh, Varshney, & Agarwal, 2016).

HCAs, PAHs and N-nitrous compounds are well-known carcinogens which are detected in processed meat products (Hasnol, Jinap, & Sanny, 2014; Jinap et al., 2013; Liao, Wang, Xu, & Zhou, 2010; Oz & Kaya, 2010; Salmon, Knize, & Felton, 1997). HCAs are mainly formed with the presence of free amino acids, carbohydrates and creatine under high cooking temperature (Rahman, Sahar, Khan, & Nadeem, 2014). IARC (1993) classified the following 5 aminoimidazoarenes (AIAs) compounds as human carcinogens, including 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4, 8-DiMeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). PAHs are hydrocarbons that contain two or more benzene rings, such as pyrene, anthracene and naphthalene. They can be formed through incomplete combustion or pyrolysis of organic components, including fat, protein and carbohydrates at the temperature over 200 °C. Grilling, roasting and smoking meat products likely contain high level of PAHs (Alomirah et al., 2011). Benz[a]anthracene (BaA) and benzo[a]pyrene (BaP) are the most potent carcinogenic PAHs in processed meat products (PHE, 2008). The metabolite of BaP, BaP-7,8-diol-9,10-epoxide, has been reported with the highest tumour-inducing activity due to causing DNA adducts (Purcaro, Moret, &

Conte, 2013).

Vegetable oils contain various antioxidants such as vitamin E, β -carotenes and phenolic compounds (Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, & de la Serrana, 2015). These antioxidants have been characterized as free radical scavengers during cooking, which might inhibit the formation of carcinogens (Janoszka, 2011; Wong, Cheng, & Wang, 2012). Cheng, Chen, and Wang (2007) reported that marinating beef patties with phenolic compounds such as epicatechin gallate, rosmarinic acid and carnosic acid could significantly reduce HCAs by 24%–70% in final cooked products. Balogh, Gray, Gomma, and Booren (2000) found that HCAs (IQ, MeIQ, MeIQx, DiMeIQx and PhIP) were inhibited by 45%–75% when sprayed 1% vitamin E (w/w) on the surface of beef patties before frying. Therefore, in the concern of the carcinogen level in processed meat products, replacing saturated fat with vegetable oils rich in unsaturated fatty acids needs to be justified. Thus, the objectives of this study were to (1) explore the effect of partially replacing pork back fat with sunflower oil, olive oil and grape seed oil on the formation of HCAs and PAHs; (2) examine the effect of different cooking temperatures on the formation of carcinogens in fat reduced pork patties.

2. Material and methods

2.1. Materials

Three batches of lean pork leg and pork back fat with 40.3% SFA, 43.4% MUFA and 10.0% PUFA (McCance & Widdowson, 2002) were purchased from Jennings Caversham (Reading, UK) at different time point to consider the batch effect. Excess visible fat on pork legs was trimmed, then minced by a Kenwood Food processor (Chef Titanium KM010, 4.6, Kenwood Limited) and vacuum packed separately. Raw materials were stored at -18°C and defrosted 24 h at 4°C before use. Commercial grape seed oil (Waitrose[®], produced in Italy) with 12.4% SFA, 20.2% MUFA, 68.2% PUFA, 10–15 mg tocopherols and 5.9–11.5 mg/100 g polyphenols (Bail, Stuebiger, Krist, Unterweger, & Buchbauer, 2008), sunflower oil (Morrison's[®], produced in UK) with 14.3% SFA, 20.5% MUFA, 63.3% PUFA and 50 mg/100g tocopherols (McCance & Widdowson, 2002) and refined olive oil (Filippo[®], phenols were removed by industrial process, produced in Italy) with 14.3% SFA, 73.0% MUFA, 8.2% PUFA and 5–300 mg tocopherols (McCance & Widdowson, 2002) were purchased from local supermarket (Reading, UK). Oils were kept in refrigerator (4°C) before making patties and further analysis.

The standards IQ (2-amino-3-methyl-imidazo [4,5-f] quinoline), MeIQ (2-amino-3,4-dimethyl-imidazo [4,5-f] quinoline), MeIQx (2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline), 4,8-DiMeIQx (2-amino-3,4,8-trimethyl-imidazo [4,5-f] quinoxaline), PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine), BaA (Benz[a]anthracene) and BaP (benzo[a]pyrene) were purchased from Toronto Research Chemicals (Toronto, Canada). Ammonium acetate, triethylamine, acetonitrile (HPLC grade), bovine serum albumin (BSA), dinitrophenylhydrazine (DNPH), ethyl acetate 99.5% 0.9000 g/ml, 6M guanidine HCl (pH 6.5), hydrochloric acid solution 0.1M, methanol (HPLC grade), HPLC grade water, sodium hydroxide 1M, perchloric acid (99.8%), sodium phosphate buffer (pH 6.5), thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were purchased from Fisher Scientific (Loughborough, UK). 2,2-Azobis(2-methylpropionamide) dihydrochloride granular 97% (ABAP), 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), (\pm)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), phosphate buffer solution 0.1 M and phosphoric acid were purchased from Sigma-Aldrich (Gillingham, UK). The solid-phase extraction Extrelut NT 20 columns and diatomaceous earth refill material were purchased

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