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Effect of green tea extract and microwave pre-cooking on the formation of heterocyclic aromatic amines in fried chicken meat products

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ABSTRACT

Heterocyclic aromatic amines (HAAs) are mutagenic compounds formed naturally in meats after thermal processing and are classified as a probable human carcinogen. Also, mutagenic potency of HAAs is about 100-fold stronger than that of aflatoxin and 2000-fold stronger than that of benzo[a]pyrene. The aims of the present study were to investigate HAA contents and to determine HAA existence in coated chicken drumsticks (CDs) and coated chicken wings (CWs) which are frequently consumed in fast food chains after purchasing from fast food restaurants, and the effects of green tea extract added into the cover material and microwave-precooking for the mitigation and the formation of HAAs in CD and CW samples produced using a laboratory model. HAA (IQx, IQ, MeIQx, MeIQ, 7,8-DiMeIQx, 4,8-DiMeIQx, PhIP, AαC, MeAαC) analysis was done by HPLC after solid-phase extraction. MeIQx is the dominant HAA in all CD and CW samples obtained from fast food restaurants, and its level was found to vary between 0.22–33.73 and 11.22–62.83 ng/g, respectively. PhIP was detected in 5 out of 20 samples from fast food restaurants with a maximum level of 3.15 ng/g, and IQx, 7,8-DiMeIQx, AαC, and MeIQ were also detected. MeIQx values of CW and CD samples produced with the laboratory model varied between not detected and 1.45 and not detected and 2.32 ng/g, respectively, while IQ, MeIQ, 7.8 DiMeIQx, 4,8-DiMeIQx, AαC, and MeAαC were not detected in any of the CW or CD samples produced using the laboratory model. HAA contents of CD and CW samples obtained from fast food restaurants were higher than those of samples produced using the laboratory model due to the possible effect of uncontrolled frying conditions in restaurants. In addition, it was determined that the addition of green tea extract (GTE) and microwave pre-cooking (MC) did not present any detectable effect on reducing the formation of HAAs in fried CDs and CWs produced using a laboratory model under controlled frying conditions.

1. Introduction

Heterocyclic aromatic amines (HAAs) are heat-induced reaction products which are identified as probable human carcinogens (Aaslyng, Duedahl-Olesen, Jensen, & Meinert, 2013; Capuano & Fogliano, 2011; IARC, 1993, 1994; Skog, Johansson, & Jägerstad, 1998). HAAs are carbon, hydrogen and nitrogen based compounds formed naturally during the cooking of protein containing foods such as meat, poultry and fish at temperatures of over 150 °C (Jägerstad, Skog, Arvidsson, & Solyakov, 1998; Oz, 2011; Persson, Graziani, Ferracane, Fogliano, & Skog, 2003). Previous researches have pointed out the relationship between high meat consumption and cancer caused by HAAs (Ferguson, 2010; Persson et al., 2003; Sugimura, 2000; Weisburger et al., 2002). In 2002, Weisburger et al. reported that there is now evidence from worldwide epidemiological studies that people who as a rule consume meat that is well cooked have a higher risk of cancer of the breast and colon. To date, more than 25 HAAs have been isolated and identified from various cooked foods (Alaejos, Ayala, Gonzáles, & Afonso, 2008; Oz, 2011). Most HAAs are highly mutagenic (Felton et al., 1984) and almost all are also carcinogenic to laboratory animals (Ito et al., 1991; Sugimura, 1995). The Maillard reaction and free radicals play an important role in the formation of HAAs. Creatine/creatinine, amino acids and sugars, which are naturally found in animal tissues, are the precursors of these HAAs (Jägerstad et al., 1983; Skog, 1993). On the other hand, the concentration and type of

Abbreviations: GTE, green tea extract; MC, microwave pre-cooking; CD, coated drumstick; CW, coated wing; HAA, heterocyclic aromatic amine; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; IQx, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; 7,8-DiMeIQx, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; AαC, 2-amino-9H-pyrido[2,3-*b*]indole; MeAαC, 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole; 4,7,8-TriMeIQx, 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline.

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HAAs are related to meat type, pH, water activity and cooking procedures such as frying, roasting, barbecuing, baking and grilling (Knize & Felton, 2005; Skog et al., 1998). Since HAAs have harmful effects on human health, it is necessary to improve the methods that can prevent the formation of HAAs in meat products (Ahn & Grün, 2005). The addition of pure antioxidants such as vitamin E or foods containing antioxidants such as sage, thyme, cherry tissue and green tea is one of the methods used to reduce the concentration of HAAs in cooked meats (Ahn & Grün, 2005; Balogh, Gray, Gomaa, & Booren, 2000; Britt, Gomaa, Gray, & Booren, 1998; Murkovic, Steinberger, & Pfannhauser, 1998; Oguri, Suda, Totsuka, Sugimura, & Waka-Bayashi, 1998; Weisburger et al., 2002; Zheng & Wang, 2001). Based on these previous studies, it could be asserted that extracts containing polyphenolic antioxidants can reduce the formation of HAAs because of their inhibitory effects on free radical reactions. Among antioxidant compounds, green tea is rich in polyphenolic content due to its catechin as a major component (Koo & Cho, 2004). Several studies have shown that the HAA inhibitory effects of green tea are due to the presence of tea polyphenols and catechins (Mukhtar, Katiyar, & Agarwal, 1994; Stavrıc, Matula, Klassen, & Downie, 1996; Yang & Wang, 1993). Oguri et al. (1998) demonstrated that among 14 kinds of antioxidants tested, green tea catechins and (−)-epigallocatechin gallate clearly suppress the formation of MeIQx, to 21% and 35% of the level of the controls, respectively. The most effective inhibition was thus observed with the addition of green tea catechins. Weisburger, Nagao, Wakabayashi, and Oguri (1994) investigated the effect of black tea, green tea, theaflavin gallate and (−)-epigallocatechin gallate on the formation of MeIQx and PhIP. This study found that green tea, black tea and the associated tea polyphenols decrease the formation or expression of the genotoxicity of HAAs in the model system (Weisburger et al., 1994).

Cooking time is another important factor that affects the formation of HAAs. Prolonged cooking time and high-temperature cooking surfaces produced the highest quantities of HAAs (Knize, Dolbeare, Carroll, Moore, & Felton, 1994; Knize & Felton, 2005). As a result, Felton, Fultz, Dolbeare, and Knize (1994) pointed out that using microwave treatment as a pre-cooking method reduced the formation of HAAs by shortening the cooking time.

Nowadays, people tend to eat ready to eat foods because of changes in daily activities and business lives. Coated chicken wings (CWs) and coated chicken drumsticks (CDs) sold in fast food restaurants have become more popular due to their short preparation time and palatability. The results of a survey performed in Turkey revealed that 70% of people consume coated chicken products in their daily lives. The vast majority of these people reported that they had begun to consume coated chicken products in recent years. Also 46% and 41% of these people prefer to consume CWs and CDs, respectively (Yaldırak & Kolsarıcı, 2012). CDs and CWs are produced by covering drumsticks and wings with batter and breader after marinating. After covering the drumsticks and wings, they are fried in a pressurized deep fat fryer for the appropriate cooking time. Thus, HAAs may be formed during the deep fat frying of meats at various levels (for example not detected–3.44 ng/g for MeIQx, not detected–0.38 ng/g for 4,8-DiMeIQx, not detected–3.44 ng/g for PhIP) (Gibis & Weiss, 2010; Jinap et al., 2013). The HAA contents of meat products such as ground beef patties, hamburger, pork, fish, chicken and duck have been calculated (Balogh et al., 2000; Janoszka, Błaszczyk, Damasiewicz-Bodzek, & Sajewicz, 2009; Johansson & Jägerstad, 1994; Klassen, Lewis, Lau, & Sen, 2002; Liao, Wang, Xu, & Zhou, 2010) but there have been limited studies on coated chicken products. In order to investigate the intake of HAAs and their risks to human health, it is important to know the HAA content in CDs and CWs sold in fast food restaurants and the reduction possibilities of HAAs in these foods. With that said, the objectives of this study were to investigate the HAA contents of CDs and CWs purchased from fast food restaurants and to determine whether HAAs were present or not and the effect of green tea extract alone or combined with microwave pre-cooking (MC) on the formation of HAAs in fried CDs and CWs produced by a laboratory model system.

2. Materials and methods

2.1. Chemicals

Petroleum ether, sulfuric acid, boric acid, sodium hydroxide (NaOH), hydrochloric acid, trichloroacetic acid (TCA), sodium chloride, di-sodium tetraborate decahydrate, sodium dodecyl sulfate, orthophthaldialdehyde, beta(2)mercaptoethanol, diethyl ether, and diacetyl, 1-naphthol were purchased from Merck Co. (Darmstadt, Germany) and picric acid, creatine standard, creatinine standard, and D-(+)-glucose (≥99.5%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). For HAA analyses, the following chemicals were purchased from Toronto Research Chemicals (Downsview, Ontario, Canada): 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3-methylimidazo[4,5-*f*] quinoxaline (IQx), 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), 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP), 2-amino-9H-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole (MeAαC), and 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline (4,7,8-TriMeIQx, as the internal standard). All other chemicals were of high performance liquid chromatography (HPLC) or analytical grade. Marinating sauce and breader were supplied from a commercial company, Holland Fried Chicken (HFC), Ankara, Turkey. Guardian green tea extract 20S was purchased from Danisco (Copenhagen, Denmark).

2.2. Coated chicken drumstick (CD) and chicken wing (CW) samples

In the first step, CDs and CWs were purchased from popular fast food restaurants in Ankara, Turkey, at 10 different times to determine whether an HAA was present or not.

In the second step, 24 h postmortem chicken drumsticks and chicken wings (approximately 20 kg each) were supplied from a commercial facility in Turkey. They were marinated with a marinating sauce and stored at 4 °C overnight. The basic ingredients of the marinating sauce were oil, salt, red pepper, black pepper, coriander, ginger, coconut, garlic, citrate, basil, cumin, rosemary and thyme. A total of 100 g of marinating sauce was used for 2 kg of drumsticks or wings. Also, marinating yield was 103.07% and 103.97% for drumsticks and wings, respectively. On the following day, 2 kg of breader including 0%, 0.5%, 1.5%, and 3% green tea extract (GTE) was prepared. Batter was made by dissolving 500 g of breader including GTE in 875 mL of water. Marinated drumsticks and wings were divided into 8 groups individually. Four of those groups were first dipped into a batter and then breader to produce the following treatments: T1) CDs-0%GTE, T2) CDs-0.5%GTE, T3) CDs-1.5%GTE, and T4) CDs-3%GTE; T1) CWs-0%GTE, T2) CWs-0.5%GTE, T3) CWs-1.5%GTE, and T4) CWs-3%GTE. The battered and breaded product yields were 111.57% and 123.97% for drumsticks and wings, respectively. After coating, drumsticks and wings were deep fat fried in sunflower oil for 8.5 min and 3.5 min, respectively, in a high pressure fryer at 175 °C under 8 psi of pressure. Frying oil was changed after frying of each group. Frying yield was 83.60% and 84.85% for CDs and CWs, respectively. Concerning the four other groups of marinated drumsticks and wings, microwave pre-cooking was used to shorten the frying time. Upon the completion of the marinating process, marinated drumsticks and wings were placed in a tray as a monolayer and microwaved (a bench top microwave, HMT84G451, Bosch, Germany) at 360 W for 7 min and 5 min, respectively. Microwave yield was 97.28% and 98.21% for drumsticks and wings, respectively. Then, they were also covered with the same batter and breader including 0%, 0.5%, 1.5%, and 3% GTE to produce the following treatments: T5) CDs-0%GTE + MC, T6) CDs-0.5%GTE + MC, T7) CDs-1.5%GTE + MC, and T8) CDs-3%GTE + MC; T5) CWs-0%GTE + MC, T6) CWs-0.5%GTE + MC, T7) CWs-1.5%GTE + MC, and T8) CWs-3%GTE + MC. Then CDs and CWs were deep fat fried for 5.5 min and 2.5 min, respectively. The battered