Meat Science 96 (2014) 1177–1184

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Influence of selected quality factors of beef on the profile and the quantity of heterocyclic aromatic amines during processing at high temperature

Arkadiusz Szterk *, Bożena Waszkiewicz-Robak

Department of Functional Food, Organic Food and Commodities, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences, 159 c Nowoursynowska, 02-776 Warsaw, Poland

ARTICLE INFO

Article history:
Received 26 August 2013
Received in revised form 16 November 2013
Accepted 20 November 2013

Keywords:
Mutagens in food
Carcinogens in food
Free amino acids
Nitrogenous compounds
Purines
Pyrimidines

ABSTRACT

New factors were identified impacting significantly on the formation of HAA during grilling. The number and profile of HAA in grilled beef depend on the fattening system (intensive and semi-intensive), and the effect of the animal's sex. The fewest HAAs were formed in rib steak from heifers from a semi-intensive fattening system. A significant effect of storage of meat in refrigerated conditions (5 to 15 days) was also demonstrated on the formation of HAA during grilling. The longer the raw meat was stored, the more HAA was formed during grilling. The quantity of HAA was strongly correlated with the content of free amino acids and a very strong correlation was found with an increasing content of free purine and pyrimidine bases and their nucleosides.

1. Introduction

The content of heterocyclic aromatic amines (HAA) in the food varies due to the fact that there are many factors, which influence their formation. It is known that the most important factors are: the temperature, the type of thermal processing, duration and type of processing, the material (beef, pork, poultry, fish, etc.),(grilling, frying etc.), the content of the substrates involved in the synthesis of HAA (sugars and amino acids), spices, and natural and synthetic antioxidants, pH and duration of storage of fresh meat prior to high-temperature processing (Alaejos & Afonso, 2011; Kondjoyan et al., in press; Oz & Kaya, 2011; Polak, Andrensek, Zlender, & Gasperlin, 2009; Polak, Dosler, Zlender, & Gasperlin, 2009; Szterk et al., 2012).

The type of meat subjected to heat processing significantly influences the HAA content. In meat with a low content of water and fat and high protein and free amino acid contents, particularly creatine, many more heterocyclic aromatic amines will form (Gu et al., 2002). Research has shown that meat with the skin attached produces less HAA than meat without skin (Chiu, Yang, & Chen, 1998; Gu et al., 2002; Solyakov & Skog, 2002). Meat fat content also has a significant impact. More HAA are formed in lean meat than in fatty meat, for several reasons. Firstly, there are fewer precursors for HAA synthesis in fatty meat (lower levels of free amino acids, glucose and protein). Secondly, fatty meat has better heat penetration than lean meat and thus cooks faster by roasting, frying and grilling (Abdulkarim & Smith, 1998; Knize, Salmon, Mehta, & Felton, 1997). Comparison of HAA content in red meat (beef, pork), poultry and fish shows that the amounts of HAAs vary in different meats. There are considerably more PhIP and IFP formed in poultry, while more MeIQx is formed in beef, pork and fish (Borgen, Solyakov, & Skog, 2001; Skog, Johansson, & Jagerstad, 1998; Skog & Solyakov, 2002).

The method of thermal processing also has an influence on the formation of HAA in food. Skog, Eneroth, and Svanberg (2003) showed that roasting in the oven with the circulation of steam (210 °C), frying in a non-fat frying pan (170 °C) and deep fat frying (170 °C) of hamburgers to achieve 70 °C in the geometric centre do not contribute substantially to the formation of HAA. Mutagenic activity of extracts from processed meat in the Ames test (TA98) was also very low or absent. Felton et al. (1992) and Felton, Fultz, Dolbeare, and Knize (1994) demonstrated that cooking in a microwave oven is not conducive to the formation of HAA. They also showed that the initial thermal processing in a microwave oven before grilling, frying or roasting contributes significantly to a reduction of HAA in the final product. Heterocyclic aromatic amines in the largest quantities are formed when meat is subjected to traditional grilling (on charcoal or gas), frying in a small amount of well-heated fat and during cooking over an open fire (Keating & Bogen, 2004; Sinha, 2002; Zimmerli, Rhyn, Zoller, & Schlatter, 2001). However, frying meat coated with the bread crumbs significantly reduces the quantity of HAA (both during frying in small and large amounts of fat) as crumbs do not contain creatinine or large

* Corresponding author. Tel.: +48 22 5937057; fax: +48 22 5937040.
E-mail address: szterkarkadiusz@gmail.com (A. Szterk).

0309-1740/$ – see front matter.
http://dx.doi.org/10.1016/j.meatsci.2013.11.019

amounts of free amino acids (Busquets, Mitjans, Puignou, & Galceran, 2008; Skog et al., 2003).

HAAs also form during smoking although the use of filters or smoke formulations and flavours reduces the amount of HAA in the final product by almost 100% (Naccari et al., 2009; Simon, de la Calle, Palme, Meier, & Anklam, 2005). It is important that after frying the meat the residue after cooking (solids) is not used for the production of sauce, because it contains many aromatic heterocyclic amines and other mutagens (Johansson, Fredholm, Bjerne, & Jagerstad, 1995; Johansson & Jagerstad, 1994; Pais, Salmon, Knize, & Felton, 1999; Skog, Augustsson, Steineck, Sternberg, & Jagerstad, 1997; Skog, Steineck, Augustsson, & Jägerstad, 1995).

HAA formation in food is influenced by divalent metal ions, especially Fe^{2+}. Research shows that HAAs are also created in reactions involving free radicals, which may be catalysed by Fe^{2+} (Johansson & Jagerstad, 1993, 1996; Skog, Solyakov, & Jagerstad, 2000). Murkovic, Steinberger, and Pfannhauser (1998) demonstrated that the iron present in myoglobin catalyses HAA. However, no such catalytic effect was found with Cu^{2+} ions (Johansson & Jagerstad, 1993).

The addition of antioxidants before thermal processing reduces the formation of HAA (Busquets, Puignou, Galceran, & Skog, 2006; Busquets, Puignou, Galceran, Wakabayashi, & Skog, 2007). Marinating meat in wine or beer, which contains large amounts of natural antioxidants (mainly polyphenols), reduces HAA in beef in the case of PhIP up to about 80%, and approx. 40% MeIQx (Melo, Viegas, Petisa, Pinho, & Ferreira, 2008).

In most studies drastic thermal processing parameters have been used (high temperature, long time and thin piece of meat). This explains why in some studies with antioxidants an insignificant decrease of HAA content was observed, and in others the HAA contents, such as PhIP, increased (Cheng, Chen, & Wang, 2007). There is little literature on the impact of the time of keeping meat after slaughter on the profile and amount of heterocyclic aromatic amines or how the type of farming and the sex of the animals from which the meat is derived affects their formation during thermal processing. There are no statistical models which correlate the amount of HAA, formed in the final product with the amount of HAA precursors, i.e. free amino acids and there are no studies, which correlate the content of nitrogen bases (purine and pyrimidine) and their nucleosides with the formation of HAA. Manabe, Suzuki, Wada, and Ueki (1993) and Manabe, Kurihara, et al. (1993), have shown that the nucleic acid consisting of purines and pyrimidines may be a substrate for the synthesis of HAA.

The object of the study was to establish the influence of some factors i.e. the cooking regime of beef, time in cold storage after slaughter on the quantity of heterocyclic aromatic amines formed. In addition, the effect of the breeding system and sex of the animals, from which the beef was obtained on the formation of HAA during the grilling, was studied. The correlation of the quantity of HAA in the final product with the content of precursors in the raw meat, such as free amino acids, nitrogenous bases (purine and pyrimidine bases) and their nucleosides was determined.

2. Material and methods

2.1. Samples

The samples consisted of 12 sirloins and 12 rib steaks from 12 steers of the Limousin (LM) breed, 12 sirloins and 12 rib steaks from 12 heifers of the same breed from semi-intensive fattening and 12 sirloins and 12 rib steaks from 12 steers, of the same breed, but from intensive fattening. The meat was collected 24 h after slaughter. Meat was purchased in Warsaw. In the market there were available heifers and steers from semi-intensive fattening and steers from intensive fattening systems. Slaughter took place in the autumn of 2012. Animals came from different farms but were produced in the beef production system in force in Poland, which aims to standardise the production of beef (QMP — Quality Meat Production).

2.1.1. A brief course of fattening

Fattening was conducted at two levels of intensity: semi-intensive fattening (1000 g feed kg^{-1} day^{-1}) and intensive fattening (1300 g feed kg^{-1} day^{-1}) to two final weights within each of the groups: steers, intensive fattening: final weight about 600 kg and age 16–17 months, steers from semi-intensive fattening: final weight about 550 kg and age 17–18 months, heifers from semi-intensive fattening: final weight about 400 kg and age 15–16 months.

2.2. Thermal treatment

2.2.1. Grilling

Three 2.5 cm-thick steaks were cut off from each sirloin and each rib steak and grilled in an electrical grill at 180 °C. One side of each steak was grilled to 35 ± 2 °C, then the steak was turned upside down and grilled until the temperature of its geometrical centre reached 70 ± 2 °C. The temperature of the geometrical centre of each steak was maintained for 5 ± 1 min before they were removed from the grill. Average steak grilling time was 10 ± 1 min.

2.3. Heterocyclic aromatic amines determination

25 g of meat was placed in a glass bottle, volume 100 ml, covered with a cap with Teflon seal, to it was added 40 ml of 1 M NaOH and 25 μl of internal standard (150 pg — 13C2 and 15 N Trp-P 1). The sample was inserted in a shaking incubator at 80 °C for approx 2 h. After the alkaline hydrolysis, the sample was cooled and mixed with 50–60 g of diatomaceous earth (Diatomaceous earth Sigma Aldrich 392545). Powdery sample was transferred to a catheter syringe with volume of 160 ml (at the bottom of it was placed a small amount of glass wool to prevent loss of the sample), and then the whole was slightly compacted to level the material. The syringe was placed in a suction flask, volume 250 ml, which was sealed and then 400 ml of ethyl acetate was extracted using a slight vacuum (700 mbar). The ethyl acetate solution was filtered through fluted filter paper containing about 15 g of anhydrous sodium sulphate to dehydrate the sample. The whole was filtered directly into a 500 ml flask, and the solvent evaporated to a volume of approx. 5 ml using a vacuum evaporator. The sample thus prepared was filtered through fluted filter paper directly into the activated OASIS MCX 150 mg, ion-exchange stanchion 6 ml (Waters), additionally fitted with a container to increase its capacity to 50 ml. The sample was washed with ethyl acetate several times, using about 40 ml of solvent. Purification of the sample and next steps of sample preparation, chromatographic separation and detection were carried on by the method described by Szterk et al. (2012) and Szterk (2013).

2.4. Free amino acids, purines, pyrimidines and nucleosides determination

Free amino acids, purines, pyrimidines and nucleosides were determined in raw meat samples in accordance with Szterk and Roszko (2013).

2.5. Statistical methods

The results were statistically analysed using Statistica 10 software (StatSoft Inc. 2012). Mean values and standard deviations (SD) were calculated. The following statistical methods were employed: hierarchical and non-hierarchical analysis, classification tree analysis, correlation analysis and analysis of variance.

Hierarchical analysis involved the use of the Ward agglomeration method, and the squared Euclidean distance was employed as the measure of distance. Criteria for the number of clusters were determined based on the course of the agglomeration process, and cluster