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Effect of High Water-Holding Capacity on the Formation of Heterocyclic Amines in Fried Beefburgers

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Mutagenic/carcinogenic heterocyclic amines (HAs) are formed in the crust during the cooking of meat. The influence of cooking loss, time, and temperature on the formation of HAs was investigated in fried beefburgers. 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx) were identified in all samples. The amounts of PhIP, MeIQx, and 4,8-DiMeIQx increased with increasing cooking time, and this effect was significant for all three HAs. The pan temperature had a significant effect on the formation of PhIP and 4,8-DiMeIQx. The addition of NaCl/sodium tripolyphosphate to the beefburgers reduced the cooking loss and decreased the formation of PhIP, MeIQx, and 4,8-DiMeIQx. This decrease was significant for MeIQx and 4,8-DiMeIQx. The results clearly show that it is possible to modify cooking practices to minimize the formation of HAs.

KEYWORDS: Cooking; heterocyclic amines; PhIP; MeIQx; fried meat; cooking loss; beefburger; water-holding capacity

INTRODUCTION

Frying is a common method of preparing meat. It is fast and easy, resulting in an appetizing surface color and pleasant aroma. Around 50% of all meat dishes in the Scandinavian countries are fried (*1*), and today there is an increased tendency to rely on industrially prepared meat dishes. During the cooking of meat and meat products, mutagenic/carcinogenic heterocyclic amines (HAs) are formed at parts per billion (ppb) levels. HAs have been shown to induce tumors in various organs in long-term studies in rats, mice, and nonhuman primates (for a review, see refs *2* and *3*). The International Agency for Research on Cancer (IARC) regards eight of the HAs tested to date as possible human carcinogens (class 2B) and one as a probable human carcinogen (class 2A) and recommends a reduced dietary intake of these compounds (*4*). More than 20 HAs have been identified in cooked foods (*5*, *6*). The most abundant HAs are 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-phenyl-imidazo[4,5-*b*]pyridine (PhIP) (*7*). The precursors for their formation are free amino acids, creatine or creatinine, and sugars (*8*), which are naturally occurring compounds in muscle tissues. Some HAs are formed via the Maillard reaction (*8*), but a free radical mechanism has also been suggested (*9*).

It is well-known that cooking temperature and time are important factors in the formation of HAs; higher temperature and longer cooking time result in larger amounts of HAs (*10*−*12*). Heat transport is another important factor for the formation of HAs (*13*). During cooking, efficient heat transport to the meat surface results in a high surface temperature, which enhances the formation of HAs. Different cooking procedures involve different types of heat transport (*14*), and thus different amounts of HAs are formed. Cooking experiments with chicken fillets have shown that frying generally produces higher amounts of HAs than other cooking methods (*15*). Other factors that influence the formation of HAs are the concentrations and relative amounts of precursors and the presence of water (*16*, *17*). Different ways of reducing the formation of HAs have been suggested, for example, adjusting recipes by the addition of ingredients with antioxidative (*18*−*20*) or water-holding properties (*21*). During cooking, the amounts of precursors at the meat surface may be enhanced by the transport of water and water-soluble precursors from the inner parts of the meat. This mass transport is essential for the formation of HAs and may be influenced by water-binding ingredients (*21*). A high cooking loss has been found to be related to the formation of large amounts of HAs (*12*, *15*, *21*, *22*).

The objective of this work was to study the relationship between cooking loss during the frying of beefburgers and the formation of HAs. Such data are important as a basis for advice to the food industry, restaurants, and consumers on how to modify cooking procedures to minimize the formation of HAs in cooked meat. Beefburgers were selected as the model food item, as they are a common meat dish in the Western diet. The study design included some very well-done samples, which is common in everyday cooking, but all beefburgers were edible.

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Table 1. Factorial Design of the Frying Experiments and Amounts of PhIP, MeIQx, and 4,8-DiMeIQx Found in the Beefburgers

sample	time (min)	temp (°C)	addition of NaCl and TPP	PhIP[a] (ng/g)	MeIQx[a] (ng/g)	DiMeIQx[a] (ng/g)
1	3.5	180	yes	0.65 ± 0.16	0.05 ± 0.002	0.15 ± 0.04
2	5.5	180	yes	2.62 ± 1.64	0.94 ± 0.15	0.71 ± 0.39
3	3.5	180	no	0.79 ± 0.27	0.61 ± 0.28	0.42 ± 0.15
4	5.5	180	no	4.07 ± 2.21	2.76 ± 0.79	1.73 ± 0.53
5	3.5	220	yes	0.90 ± 0.57	0.29 ± 0.16	0.34 ± 0.12
6	5.5	220	yes	4.78 ± 3.77	1.63 ± 0.66	1.58 ± 0.48
7	3.5	220	no	2.78 ± 1.62	1.16 ± 0.74	0.63 ± 0.34
8	5.5	220	no	8.08 ± 3.75	2.36 ± 0.18	1.62 ± 0.42

[a] Concentrations are given as ng/g of cooked beefburger ($n = 4$).

MATERIALS AND METHODS

Chemicals. Solvents and chemicals were of HPLC or analytical grade. Water was passed through a Milli-Q water purification system (Millipore, Bedford, MA). The following HAs were used as reference compounds: 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine (DMIP), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 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), 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) and were purchased from Toronto Research Chemicals (Toronto, Canada). The chemical purity of the synthetic references was >99%, according to the manufacturers. This was confirmed using HPLC (*22*) with UV detection for each of the reference compounds. A mixture of the different HAs in MeOH (2 ng of each compound/μL) was used as a spiking mixture. Chemicals used for analysis of creatine and creatinine were purchased from Roche Diagnostics Scand AB (Bromma, Sweden). Sodium tripolyphosphate (TPP) was obtained from Labora Chemicon (Sollentuna, Sweden). The following materials were used for solid-phase extraction: diatomaceous earth (Isolute), obtained from Sorbent AB (Västra Frölunda, Sweden) and PRS and C_{18} columns (Varian), from Scantech Lab (Partille, Sweden).

Beefburgers. Minced meat from beef (M. pectoralis superficialis and M. pectoralis profundus) was purchased from a local store. According to the label, the fat content was 10%. Preliminary experiments were carried out to study how the cooking loss was affected by various water-binding ingredients that are commonly used in the food industry. The meat was then divided into two parts: one part was mixed with water containing 1.5% NaCl and 0.3% TPP (1.0 mL of water/100 g of meat), and the other part was mixed with water containing no additives. Each part was mixed in a domestic mixer for 1 min and formed into patties (87 mm in diameter, 10 mm in thickness, each weighing 90 g) using a special punch. The beefburgers were covered with aluminum foil and stored in the refrigerator (6 °C) overnight before the frying experiments.

Experimental Design. A pilot study was performed to determine the range of cooking times and temperatures resulting in beefburgers with a center temperature of at least 71 °C and a brown, appetizing surface color. The frying experiment was performed in a 2^3 factorial design. On the basis of the results of the pilot study, the pan temperature was set to 180 °C (low) or 220 °C (high) and the frying time to 3.5 min (low) or 5.5 min (high). The beefburgers were prepared with and without the addition of NaCl and TPP. This gives in total eight combinations, as summarized in **Table 1**. Four beefburgers were made for each combination. The beefburgers were allowed to reach room temperature (20 °C) and were then fried in a double-sided, thermostat-controlled, Teflon-coated square frying pan, 235 × 235 mm. No fat was used for frying. Four beefburgers at a time were placed in the frying pan when the pan surface had reached the selected temperature. Three K-type thermocouples (0.6 mm) were used to register the temperature in one of the beefburgers during each frying session, one thermocouple was inserted into the center of the beefburger and two were placed in the spaces between the pan and the upper and lower surface of the beefburger. The thermocouples were connected to a data logger, and the temperature was recorded every 5 s. Each beefburger was weighed before and after frying to determine the cooking loss during frying. The crust was cut off with a scalpel, freeze-dried, and stored in the freezer at −18 °C until analysis.

Extraction of HAs. The crust from each beefburger was subjected separately to extraction and purification of HAs, according to the solid-phase extraction method of Gross and Grüter (*23*) with slight modifications (*24*). Briefly, ~3 g of freeze-dried sample was dissolved in 1 M NaOH and mixed with diatomaceous earth and then transferred to empty columns. Ethyl acetate was used as extraction solvent. The eluate was passed through PRS columns and C_{18} columns. The final eluate was evaporated to dryness, and the residues were then dissolved in MeOH. Extraction recovery rates for the different HAs were determined by the addition of 100 μL of spiking mixture to one sample extracted in parallel with four unspiked samples.

Identification and Quantification of HAs. The HAs were identified and quantified by LC-MS as described previously (*25*). Liquid chromatography was performed with a Zorbax SB-C8 (150 mm × 4.6 mm) column, and the eluent phase was a combination of water (pH adjusted to 3.5 with acetic acid) and acetonitrile. The mass detector was an ion-trap mass detector, LCQ^{DECA} from Thermo Finnigan (San Jose, CA) with an electrospray ion source (ESI), and single-ion monitoring (SIM) was performed. The HAs were quantified by using peak areas, and the results were corrected for incomplete recovery.

Analysis of Creatine, Creatinine, and Moisture. The crumb and raw meat were analyzed for creatine and creatinine with an enzymatic method (Boehringer Mannheimer) as described earlier (*26*). The measurements were made in duplicate. The absorbance was measured using a UV spectrophotometer (Perkin-Elmer, Lambda 10). The moisture content was measured in duplicate in the crust and crumb of the beefburgers and in the raw meat by gravimetric determination, that is, drying at 105 °C to constant weight (~24 h). The outer layer (2 mm thick) was denoted as the crust and the rest as the crumb. Samples of the crumb were taken from the center of the beefburgers.

Statistics. The experiment had a full 2^3 factorial design with four replicates. Data were evaluated using a computer program, Minitab (*27*), to elucidate whether the results of the experiments differed significantly for the different factors. The effects of temperature (T), time (t), addition of NaCl and TPP (A), the two-factor interactions temperature−time ($T \times t$), temperature−additive ($T \times A$), and time−additive ($t \times A$), and the three-factor interaction temperature−time−additive ($T \times t \times A$) were analyzed.

RESULTS

Concentrations of Heterocyclic Amines. PhIP, MeIQx, and 4,8-DiMeIQx were the most abundant HAs and were identified in all samples at both temperatures, the concentrations being 0.1−2.4, 0.2−1.6, and 0.6−8.0 ng/g of uncooked weight, respectively. The concentrations of PhIP, MeIQx, and 4,8-DiMeIQx at each frying conditions are given in **Table 1**. (These values have been corrected for incomplete recovery.) DMIP was detected in all of the samples fried at 220 °C, at levels ranging from 0.6 to 2.0 ng/g, but not in the samples fried at 180 °C.

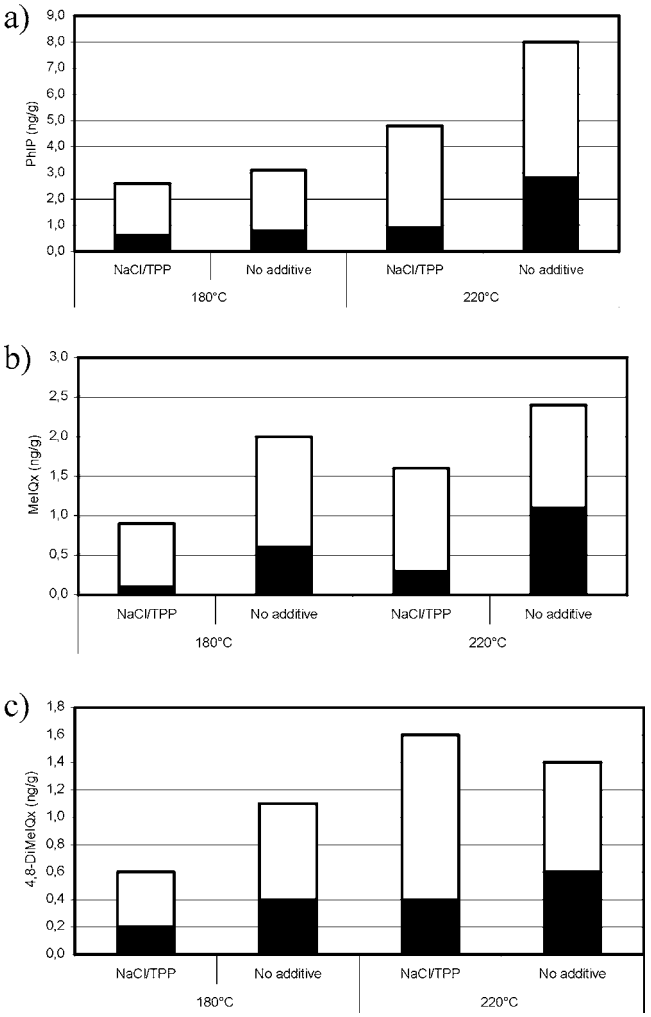

Figure 1. Total amount of HAs (ng/g of uncooked weight) in beefburgers fried for 3.5 (black area) and 5.5 min (white area): (a) PhIP; (b) MeIQx; (c) 4,8-DiMeIQx.

Peaks at the retention times of IQ, 7,8-DiMeIQx, Trp-P-1, and Trp-P-2 were seen in some of the samples. MeIQ, AαC, and MeAαC were not detected in any of the samples. The extraction recovery rates for IQ, MeIQ, MeIQx, 4,8-DiMeIQx, and 7,8-DiMeIQx were 50−60% and those for PhIP and DMIP around 40 and 20%, respectively. For the less polar HAs, Trp-P-1, Trp-P-2, AαC, and MeAαC, recoveries were low, ∼15%.

Figure 1 shows the total amounts of PhIP, MeIQx, and 4,8-DiMeIQx formed during frying of beefburgers for 3.5 and 5.5 min, at low and high temperatures with and without the addition of NaCl and TPP. The shaded parts of the bars correspond to the short cooking time (3.5 min). Frying at 220 °C resulted in larger amounts of PhIP, MeIQx, and 4,8-DiMeIQx than frying at 180 °C. Larger amounts of the three HAs were formed during the last 2 min than during the first 3.5 min. The addition of NaCl and TPP decreased the formation of PhIP, MeIQx, and 4,8-DiMeIQx in all of the experiments except for 4,8-DiMeIQx when the beefburger was fried at high temperature for a 5.5 min.

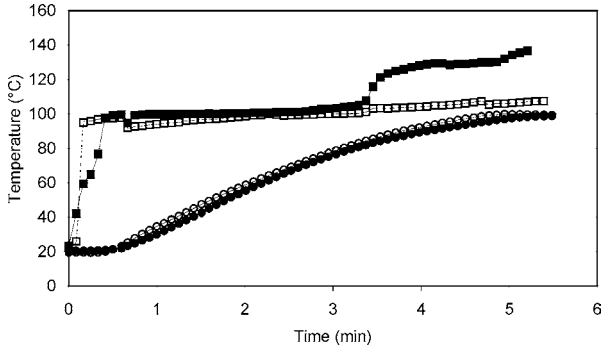

Figure 2. Temperature profile during frying of beefburgers at 220 °C for 5.5 min: (●) center and (■) surface temperatures for beefburgers with the addition of NaCl and TPP; (○) center and (□) surface temperatures for beefburgers with no additives.

The results of the factorial analysis are summarized in **Table 2**. The effect of increasing the cooking time (*t*) was significant for all three HAs. The temperature (*T*) had a significant effect on the formation of PhIP and 4,8-DiMeIQx. The addition of NaCl and TPP (*A*) significantly decreased the formation of MeIQx and 4,8-DiMeIQx, and this effect was almost significant for PhIP. Furthermore, the formation of 4,8-DiMeIQx was significantly affected by the interaction factors $T \times A$ and $T \times t \times A$. An interaction effect indicates that the effects of the factors are dependent on each other.

Temperatures. Figure 2 shows the temperature profiles recorded during two frying experiments at 220 °C, one beefburger without and one with the addition of NaCl and TPP. After 3.5 min, the surface temperature of the beefburger containing NaCl and TPP increased rapidly to 120−140 °C, whereas the surface temperature of the beefburger without NaCl and TPP remained at 100 ± 5 °C. The final center temperatures were between 75 and 100 °C (and are given in **Table 3**).

Moisture Content. The moisture content varied between 49.2 and 70.8% in the crumb and between 49.2 and 61.2% in the crust. The moisture content in the raw meat was 75%. **Figure 3** shows the relationship between cooking loss during frying and moisture content in the crust and crumb.

Cooking Loss during Frying. The cooking loss during frying was defined as the weight difference of the beefburgers

Table 2. Factorial Analysis of the Effect of Different Parameters on the Formation of PhIP, MeIQx, and 4,8-DiMeIQx in Fried Beefburgers[a]

effect parameter	PhIP (ng/g)	*F*	*P* [b]	MeIQx (ng/g)	*F*	*P*	4,8-DiMeIQx (ng/g)	*F*	*P*
main effect									
temperature (*T*)	1.04	2.53*[c]	0.019	0.13	1.61	0.122	0.17	3.49*	0.002
time (*t*)	1.80	4.34*	<0.001	0.70	8.26*	<0.001	0.42	8.9*	<0.001
addition of NaCl and TPP (*A*)	−0.84	2.04	0.054	−0.50	5.92*	<0.001	−0.15	3.24*	0.004
two-factor interactions									
$T \times t$	0.49	1.18	0.251	−0.06	−0.73	0.474	0.06	1.32	0.201
$T \times A$	0.45	1.08	0.293	−0.09	−1.15	0.262	−0.13	−2.87*	0.009
$t \times A$	0.33	0.81	0.424	0.14	1.68	0.108	0.01	0.36	0.722
three-factor interaction									
$T \times t \times$ A	0.01	0.03	0.980	−0.17	−2.05	0.052	−0.14	−2.93*	0.008

[a] The first column for each compound gives the average contrast for the different factors ($n = 4$). [b] Significance level of *F*. [c] An asterisk (*) denotes a significant value, $P < 0.05$.

Table 3. Final Center Temperatures and Ratio between Creatinine and Total Creatine and Creatinine Content in the Fried Beefburgers

	end center temp (°C)		creatinine of total creatine + creatinine (%)	
time and temp	with NaCl and TPP	no additives	with NaCl and TPP	no additives
3.5 min, 180 °C	78	85	9.7	14.9
5.5 min, 180 °C	100	100	16.9	24.1
3.5 min, 220 °C	83	75	9.5	15.1
5.5 min, 220 °C	100	95	16.4	28.7

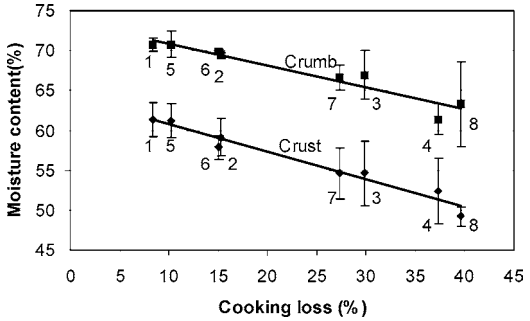

Figure 3. Relationship between cooking loss (%) and moisture content (%) in the crust and crumb of fried beefburgers. Numbers in the figure refer to the sample numbers in **Table 1**. Numbers 1, 2, 5, and 6 are beefburgers to which NaCl and TPP were added, and numbers 3, 4, 7, and 8 are beefburgers without the addition of NaCl and TPP. The values are mean values of four replicates, and the standard deviation is <1%.

before and after frying and varied from 8.4% for beefburgers with the addition of NaCl and TPP fried at 180 °C for 3.5 min to 39.6% for the beefburgers without additives fried at 220 °C for 5.5 min. **Figure 4** shows the cooking losses for the different frying conditions. The shaded parts of the bars correspond to the shorter cooking time. Most of the cooking loss appears during the first frying period, 0−3.5 min. The temperature had a minor effect on the cooking loss. The addition of NaCl and TPP had a considerable effect on the cooking loss, being more than double that in the beefburgers without the addition of NaCl and TPP.

Creatine and Creatinine Content. The concentrations of creatine and creatinine in the minced raw meat were 103 and 2.3 μmol/g of dry matter, respectively. During frying, creatine was converted to the more water-soluble form creatinine. The ratio of creatinine to total creatine and creatinine content in the crumb is given in **Table 3**.

DISCUSSION

The beefburgers in this investigation can be regarded as representative of the variations in household cooking, restaurants, and fast food outlets. The final center temperature of the beefburgers varied between 75 and 100 °C and was thus well above accepted safety limits. Consequently, the results of this study can be considered a guide for the formation of HAs during the cooking of beefburgers.

Because PhIP, MeIQx, and 4,8-DiMeIQx were detected in all samples, these compounds were selected for the statistical analysis (**Table 2**). As shown in **Figure 1**, the trends were the same for PhIP, MeIQx, and 4,8-DiMeIQx formation: the amounts were greater at higher temperature and longer frying time and in beefburgers without additives. Statistical evaluation showed that the three factors had different effects on the formation of these three HAs. The largest effect was seen when

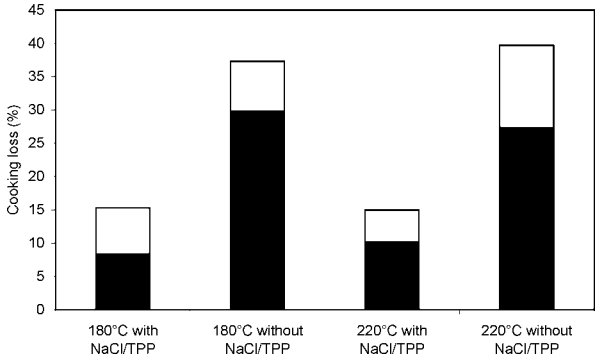

Figure 4. Cooking loss (%) during the first 3.5 min (■) and the last 2 min (□) of frying.

cooking time (*t*) was increased from 3.5 to 5.5 min, and this effect was significant for all three compounds. An increase in pan temperature (*T*) from 180 to 220 °C led to a significant increase in the formation of PhIP but not in the formation of MeIQx. Model experiments have previously shown that PhIP has a higher activation energy than MeIQx (*26*). A higher enthalpy implies that the temperature will have a greater impact on the amount formed, which is in agreement with our results. The third factor, the addition of NaCl and TPP (*A*), reduced the formation of these three HAs, the effect being significant for MeIQx and almost significant for PhIP. Addition of NaCl and TPP to the beefburgers reduced the cooking loss during frying and decreased the transport of water and water-soluble precursors to the surface, which may explain the lower amounts of HAs formed. An indication of this is the higher moisture content in the crumb of the NaCl- and TPP-containing beefburgers compared to the controls (**Figure 3**). The concentration of 4,8-DiMeIQx was lower than that of PhIP and MeIQx and showed only small variations between the different frying conditions (**Figure 1**), which complicated the statistical evaluation. The formation of 4,8-DiMeIQx was significantly affected by *T*, *t*, and *A*, but the large two- and three-factor interaction effects made it difficult to draw any general conclusions regarding the formation of 4,8-DiMeIQx. For example, the effect of temperature was dependent on the addition and NaCl and TPP.

This investigation shows that time and temperature have a considerable impact on the formation of HAs, as has been shown in earlier studies (*11*, *12*). PhIP formation has also been shown to increase at high temperatures in grilled chicken and fried beefburgers (*11*, *15*, *28*). DMIP was detected only at the higher temperature. This suggests that the formation of DMIP, which is structurally related to PhIP, is also favored by high temperature. Few studies report the concentration of DMIP in meat and model system (*16*, *17*, *29*), and less is known about its formation, but creatine and the amino acid threonine have been suggested as tentative precursors for DMIP (*16*).

Figure 3 shows a clear correlation between the cooking loss and moisture content in both the crust and the crumb. Higher cooking loss resulted in lower moisture content. When NaCl and TPP were added to the beefburgers, the cooking loss was <15%, but without NaCl and TPP, the cooking loss was >25% for all of the beefburgers, which demonstrates the water-holding effect of NaCl and TPP. Cooking loss consists mainly of fat and water in which molecules such as amino acids, creatine, creatinine, and sugars are dissolved. However, in low-fat beefburgers, the contribution of fat is small and independent of temperature (*30*). Approximately two-thirds of the total cooking loss in our study occurred during the first 3.5 min of frying, when the center temperature rose to >50 °C. When proteins

are denatured due to increased temperature, their water-holding capacity decreases and water is released from the meat (*31*). Such temperature-induced changes in water-holding capacity occur in two phases. Most of the water is released during the first phase, between 30 and 50 °C. This is due to denaturation of myofibrilar proteins, which constitute 70% of the lean meat, and most of the water in the meat is located here. The second water-loss phase occurs mainly between 65 and 90 °C and is due to similar changes in the connective tissue.

In the food industry, a mixture of NaCl and TPP is often added to meat products to improve texture, taste, and water-holding capacity, which are of great financial importance (*32*). It has been suggested that the main mechanism behind the improved water-holding capacity induced by NaCl is that Cl^- ions bind to the myofibrilar filaments in the meat, increasing the electrostatic repulsive force between them, leading to a larger myofibrilar volume that can retain more water (*33*). Phosphates are believed to increase the water-holding capacity by hydrophobic interactions, involving mainly myosin, and by increasing the pH and the ionic strength (*34*). If more water is retained inside the beefburger, less energy is needed for evaporation of water at the surface and, therefore, more energy is available for heating the surface, leading to a higher surface temperature. This can be seen in **Figure 2**, where the surface temperature of the beefburger containing NaCl and TPP increases more than the surface temperature of the beef burgers without NaCl and TPP. If this phenomenon were dominating, high retention of water would favor the formation of HAs due to the higher surface temperature. The opposite seems to be the case. More of the HAs were formed during the second period, 3.5−5.5 min, when the cooking losses were lower. One reason could be that during this period, the concentration of precursors at the meat surface had increased due to the transport of water to the surface, and the surface now starts to dry out. A high cooking loss and thus considerable transport of precursors to the surface are more favorable for HA formation than retention of water and increased surface temperature.

The contents of creatine and creatinine in the raw meat are comparable to other published results (*35*, *36*) and were measured to ensure the quality of the minced meat. Because the concentration of creatine is relatively constant in lean meat and higher than in connective tissue, the concentration of creatine could be used as an index of meat quality in minced meat products (*37*). In the beefburgers fried for 5.5 min, more creatine was converted to creatinine than in the beefburgers fried for 3.5 min, which is in accordance with literature data (*38*, *39*). No effect of the temperature was observed, probably because the center temperatures were almost the same at both frying temperatures. When the center temperature was measured during double-sided frying of beefburgers at different pan temperatures (140−220 °C), no variation in center temperatures between different pan temperatures was seen (*30*). The addition of NaCl and TPP seemed to inhibit the conversion of creatine to creatinine during frying. This observation is new. The addition of NaCl and TPP reduced the amount of creatine and creatinine at the surface because of the decreased cooking loss and at the same time reduced the concentration of creatine and creatinine as creatine is less water soluble than creatinine and less transported to the surface.

In conclusion, the formation of HAs is affected by time, temperature, and the addition of NaCl and TPP. Careful control of the cooking time may be an effective way of reducing the amount of HAs in fried beefburgers. A lower cooking temperature decreases the amount of HAs, and the time needed is, in practice, independent of the cooking temperature. Most of the cooking loss takes place during the beginning of the frying, whereas most of the HAs are formed during a later period. The addition of NaCl and TPP to beefburgers improves the water-holding capacity, and it is an effective way of reducing cooking loss and probably also the amounts of HAs formed during frying.

ABBREVIATIONS USED

HAs, heterocyclic amines; DMIP, 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine; PhIP, 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; IQx, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline; 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; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; AαC, 2-amino-9*H*-pyrido[2,3-*b*]indole; MeAαC, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; TPP, sodium tripolyphosphate; dm, dry matter.

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