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Addition of Various Carbohydrates to Beef Burgers Affects the Formation of Heterocyclic Amines during Frying

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The influence of the addition of carbohydrates with different physicochemical properties on weight loss and formation of heterocyclic amines (HAs) during the frying of beef burgers was examined. Furthermore, the capability of carbohydrates to bind HAs was tested. Beef burgers containing 1.5% NaCl and 0.3% tripolyphosphate (reference), with the addition of 1.5% carbohydrate, were fried for 5 min at 200 °C in a double-sided pan fryer. The beef burgers were analyzed for HAs with solid phase extraction and liquid chromatography/mass spectrometry. 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-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (PhIP), and 9H-pyrido[3,4-*b*]indole (Norharman) were detected in all of the beef burgers. The addition of carbohydrates affected both the weight loss and the formation of HAs during cooking. The formation of HAs could be correlated to depend on both the weight loss and the type of the added carbohydrate. Of the 11 different carbohydrates tested, raw potato starch was most capable of inhibiting the formation of HAs, while potato fiber gave the lowest weight loss and a comparably low amount of PhIP. Wheat bran and potato fiber were found to reversibly bind HAs. It is concluded that adding small amounts of certain carbohydrates may be a simple and effective way of reducing the amount of HAs and can easily be applied in households and commercial preparations of beef burgers.

KEYWORDS: Heterocyclic amines; beef burger; frying; carbohydrates; water holding; PhIP; MeIQx; 4,8-DiMeIQx; Harman; Norharman

INTRODUCTION

The intake of meat is a dietary factor considered to cause a significant number of cases of cancer (*1*), especially cancers of the esophagus, liver, urinary, and colon. Several epidemiological studies suggest an association between meat intake and tumor development (*2*, *3*). In some studies, this association is considered to be due to the presence of heterocyclic amines (HAs) in cooked meat (*4*−*7*). However, other studies find no correlation between the HAs and the incidence of cancer (*8*), thus indicating a need for further clarification about the effects of the intake of HAs on human health.

HAs are mutagenic compounds formed during the cooking of meat and meat products and have been shown to be carcinogenic in rodents (*9*). Today, around 20 different HAs are known, the most common ones being 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). These substances are formed from precursors naturally present in meat, through a reaction between creatine, glucose, and free amino acids (*10*, *11*). Temperature, time, fat, antioxidants, and heat and mass transfer are important parameters that affect the formation (*12*).

The addition of carbohydrates to meat may offer a simple, effective way to reduce the formation of HAs in meat and meat products, one which has not yet been investigated to any greater extent. Except for an interest of adding carbohydrates to decrease the formation of HAs, there is an increased interest in the use of carbohydrates to improve textural characteristics such as tenderness, juiciness, and cooking loss, as well as taste and aroma in meat products. Carbohydrates often used in this respect are different starches such as potato, tapioca, and maize starch; different gums including carrageenan, guar gum, and xantan gum; and different fibers, for example, potato fiber, beet fiber, wheat fiber, and oat fiber (*13*−*16*). The physicochemical properties of the carbohydrates, e.g., the degree of polymerization, degree of branching, and solubility, may influence the interactions between carbohydrates and proteins in meat and thus also the water-holding capacity of meat and the formation of HAs.

The effect of water holding on the formation of HAs has been studied previously where the high water-holding ability of beef

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burgers was shown to lead to a decreased formation of HAs (*17*). However, in that study, only NaCl and tripolyphosphate (TPP) were used to modify the water-holding capacity of the meat. In another study, the addition of powdered milk, golden bread crumbs, and potato starch to beef patties has been shown to lead to a decrease in both weight loss during cooking and in the formation of mutagens (*18*). When 1.5% fructooligosaccharides (FOSs), galactooligosaccharides, isomaltooligosaccharides, or inulin were added to beef burgers fried at 225 °C for 10 min, they inhibited the formation of PhIP, MeIQx, and DiMeIQx by 46−54% (*19*). However, there is a need to investigate the effect of other carbohydrates commercially used in the industry today and other potential carbohydrates and to perform the frying experiments using cooking conditions relevant for households, restaurants, and the food industry.

Several epidemiological studies have shown that dietary fibers have a protective effect against the development of cancers in the gastrointestinal tract (*3*). Thus, it is important to investigate the binding ability of HAs, which have the capacity to decrease the absorption of these compounds from the colon. Lignin has in previous studies been suggested to be responsible for the considerable binding of HAs to wheat bran and sorghum (*20*, *21*). The binding of HAs has also been shown to be higher to insoluble fibers in complex fiber mixtures (*21*).

The aim of the present investigation was to study the influence of the addition of carbohydrates on weight loss during ordinary cooking and the formation of HAs as compared with beef burgers only containing NaCl and TPP. Beef burgers were prepared with the addition of 11 different carbohydrates varying in, e.g., solubility, molecular weight, and degree of branching. Beef burgers were chosen since they are among the most common fried meat dishes in the Western diet (*22*−*24*). In addition, the binding capacity of the polysaccharides toward HAs was investigated in order to ensure correct analysis and predict the bioavailability of the HAs in the gastrointestinal tract.

MATERIAL AND METHODS

Chemicals. Solvents and chemicals were of high-performance liquid chromatography (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: PhIP, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx (2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline), Harman (1-methyl-9H-pyrido[3,4-*b*]indole),Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole), Trp-P-2 (3-amino-1-methyl-5H-pyrido[4,3-*b*]indole), AαC (2-amino-9H-pyrido[2,3-*b*]indole), and MeAαC (2-amino-3-methyl-9H-pyrido[2,3-*b*]indole). These were purchased from Toronto Research Chemicals (Toronto, Canada). Norharman (9H-pyrido[3,4-*b*]indole) was purchased from Aldrich (Steinhem, Germany). The chemical purity of the synthetic references was higher than 99%, according to the manufacturers. This was confirmed using HPLC 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. Caffeine was obtained from Sigma (Stockholm, Sweden). 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), obtained from Scantech Lab (Partille, Sweden). The tested carbohydrates were pectin, wheat bran, guar gum, methylcellulose, high amylose cornstarch, potato fiber, raw potato starch, and four FOSs: A, B, C, and D. The composition of the added carbohydrates is summarized in **Table 1**.

Meat Samples. Minced meat from beef (*M. pectoralis superficialis* and *M. pectoralis profundus*) from a local store was purchased. The fat content was 10% according to the label. A 0.2 g amount of TPP, 1.5 g of NaCl, and 1.0 g carbohydrate per 100 g meat were added. The tested carbohydrates were as follows: FOSs (FOS A, FOS B, FOS C, and FOS D), complex dietary fibers (wheat bran and potato fiber), starches (high amylose cornstarch and raw potato starch), and purified dietary fiber (pectin, guar gum, and methylcellulose). One beef burger was prepared with only TPP and NaCl (reference burger), and one was prepared without any additives (ordinary burger). Each combination 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 beef burgers were covered with aluminum foil and stored in the refrigerator (+6 °C) overnight before the frying experiments.

Table 1. Description of the Chemical Components of the Different Carbohydrates According to the Manufacturer and/or Literature

carbohydrate	chemical components/structure
FOS A	fructose and glucose
FOS B	fructose and glucose
FOS C	fructose and glucose
FOS D	fructose and glucose
wheat bran	16% protein, 23% starch, 6% fat, 25% arabinoxylans, 18% cellulose, 7% lignin, 5% soluble dietary fiber fraction[a]
potato fiber	5% protein, 12% starch, 21% arabinoxylans 14%-pectin, 23% cellulose, 2% lignin, 30% soluble dietary fiber fraction[b]
raw potato starch	64% resistant starch, amylose/amylopectin 20/80[b]
HiMaize	59% resistant starch, amylose/amylopectin 80/20[b]
methylcellulose	polymer of glucose, β-linkages, substituted methyl groups
pectin	polymer of glucose, galacturonic acid, rhamnose, and galactose
guar gum	polymer of mannose and galactose, galactomannan

[a] Ref *40*. [b] Ref *32*.

Frying. The beef burgers were allowed to reach room temperature (20 °C) and were then fried in a double-sided, thermostat-controlled, Teflon-coated, square frying pan, 235 mm × 235 mm, at 200 °C for 5 min. The pan surfaces were coated with margarine. Thermocouples were connected to a data logger, and the temperature of the frying pan was recorded every 10 s. Each beef burger was weighed before and after frying to determine the weight loss during cooking. The crust (approximately 2 mm and 30 ± 4 g/burger) was cut off with a scalpel, freeze-dried, and stored in the freezer at −18 °C until analysis.

Binding of HAs to Carbohydrates in Vitro. In a first set of experiments, the capability of carbohydrates to bind HAs was investigated; 20 mg of each polysaccharide was dissolved in 1 mL of water together with 100 μL of a reference solution containing 2 ng/μL of 10 different HAs (**Table 2**). The mixture was incubated and shaken at 37 °C for 1 h and thereafter centrifuged 5 min at 3000 rpm. The supernatant was collected and injected into a HPLC system (Varian 9010 Liquid Chromatograph). The column (ODS 80 TosoHaas, 250 mm × 4.6 mm i.d., 5 μm, Labkemi, Lund, Sweden) was eluted with acetonitrile and 0.01 M triethylamine (pH adjusted to 3.6 with acetic acid). The flow rate was 1.0 mL/min, and the injection volume was 90 μL. Chromatograms and UV spectra were obtained using a photodiode array UV detector (Varian 9065, Polychrome) and a fluorescence detector (Varian 9070). HAs were identified by comparing UV spectra and retention times with those from the reference solution run under the same conditions. MeIQx and 4,8-DiMeIQx were quantified using data from the UV detector. PhIP, Harman, and Norharman were quantified using fluorescence data. Binding was calculated as the ratio between the peak area of the supernatant and the reference solution. To examine if the HAs were reversibly bound to the carbohydrates and thus not affecting the extraction, the solid phase extraction of HAs mentioned in the previous section was performed with the mixture of HAs solution and the two carbohydrates that were found capable of binding HAs.

Extraction and Analysis of HAs in Beef Burgers. The beef burgers were extracted using the solid phase extraction method developed by Gross and Grüter (*25*) with slight modification (*26*). Briefly, samples were homogenized in 1 M NaOH and mixed with diatomaceous earth. The HAs were extracted with ethyl acetate and adsorbed to cartridges containing propylsulfonic acid silica (PRS), a strong cation exchanger. The apolar HAs were eluated and adsorbed to C_{18} cartridges using 0.1 M HCl, 60:40 v/v, 0.1 M HCl:MeOH, and water. The polar HAs were

Table 2. Amount of HAs in Beef Burgers Expressed Both as ng/g Uncooked and Cooked Beef Burger[a]

		MeIQx		PhIP		Norharman		total
	cooking loss (%)	uncooked	cooked	uncooked	cooked	uncooked	cooked	cooked
ordinary	45.9	0.3 ± 0.2	0.6 ± 0.3	23.9 ± 19.8	44.2 ± 36.6	8.3 ± 0.6	15.3 ± 1.1	60.1 ± 38.1
reference	25.5	0.1 ± 0.0	0.1 ± 0	8.2 ± 2.6	11.0 ± 3.5	3.8 ± 0.3	5.1 ± 0.4	16.2 ± 4.0
guar gum	38.1	0.7 ± 0.3	1.1 ± 0.5	5.4 ± 2.8	8.7 ± 4.5	4.8 ± 1.8	7.8 ± 3.0	17.6 ± 8.0
pectin	42.7	0.3 ± 0.1	0.5 ± 0.2	9.8 ± 3.2	17.1 ± 5.5	9.6 ± 3.3	16.8 ± 5.7	34.4 ± 11.5
methylcellulose	36.2	0.9 ± 0.1	1.4 ± 0.2	4.9 ± 0.2	7.7 ± 0.4	3.6 ± 0.4	5.6 ± 5.2	14.7 ± 5.7
HiMaize	25.1	0.3 ± 0.1	0.4 ± 0.1	7.6 ± 5.7	10.1 ± 7.7	10.4 ± 3.6	13.9 ± 0.5	24.4 ± 8.3
potato starch	36.0	0.1 ± 0.1	0.2 ± 0.2	1.9 ± 1.0	3.0 ± 1.5	1.5 ± 1.1	2.3 ± 5.7	5.5 ± 7.4
potato fiber	25.2	0.1 ± 0.1	0.1 ± 0.1	4.5 ± 1.8	6.0 ± 2.4	3.1 ± 1.7	4.1 ± 1.5	10.2 ± 4.0
wheat bran	28.2	0.2 ± 0.2	0.3 ± 0.2	5.3 ± 2.5	7.4 ± 3.4	4.0 ± 0.7	5.6 ± 2.3	13.3 ± 5.9
FOS A	43.4	4.0 ± 1.2	7.1 ± 2.1	6.7 ± 1.9	11.8 ± 3.3	7.0 ± 1.0	12.4 ± 1.3	31.3 ± 6.7
FOS B	44.8	13.5 ± 1.3	24.5 ± 2.4	6.1 ± 2.2	11.1 ± 4.0	3.8 ± 1.0	6.9 ± 1.8	42.5 ± 8.2
FOS C	31.4	3.7 ± 1.2	5.4 ± 1.8	5.0 ± 2.4	7.3 ± 3.6	2.7 ± 0.8	3.9 ± 1.1	16.6 ± 6.4
FOS D	30.9	0.4 ± 0.1	0.6 ± 0.1	4.1 ± 0.6	5.9 ± 0.8	1.0 ± 0.4	1.4 ± 0.5	7.9 ± 1.5

[a] Ordinary beef burger denotes beef burger without any additives, and reference beef burger denotes beef burger with NaCl and TPP but no carbohydrate. Mean value ± SD.

eluated with ammonium acetate and adsorbed to C_{18} cartridges. The samples were finally eluated from the C_{18} cartridges by 9:1 MeOH:NH_3. 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 three unspiked samples.

The samples were analyzed using a liquid chromatography/mass spectrometry (LC/MS) system as described previously (*27*). The extracts were evaporated to dryness under nitrogen and thereafter dissolved in 100 μL of caffeine solution to correct for variations in injection volume. HPLC analysis was performed with a Zorbax SB-C8 (150 mm × 4.6 mm, i.d. 5 μm, Agilent Technologies, Palo Alto, CA) column. The eluent phase was a combination of water (pH adjusted to 3.5 with acetic acid) and acetonitrile. An ion trap mass detector (LCQDECA from Thermo Finnigan, San José, CA) with an electrospray ion source was used, and selected ion monitoring was performed. The HAs were quantified by using peak areas, and the results were corrected for incomplete recovery. Each extract was injected in duplicate.

RESULTS

Formation of HAs. The amounts of HAs in the fried beef burgers, expressed both as ng/g raw meat and ng/g cooked meat, are presented in **Table 2**. MeIQx, 4,8-DiMeIQx, PhIP, and Norharman were identified in all samples. Because of coeluting compounds, Harman could not be quantified. In the ordinary beef burgers (without additives), the concentrations of MeIQx, PhIP, and Norharman were 0.6, 44.2, and 15.3 ng/g cooked meat, respectively. For beef burgers containing NaCl and TPP with and without carbohydrates, the concentrations of MeIQx, PhIP, and Norharman varied between 0.1 and 24.5, 3.0 and 17.1, and 2.3 and 16.8 ng/g cooked meat, respectively. PhIP and Norharman were the most abundant HAs. Examining the data in **Table 2**, expressed in ng HA per g cooked meat, it is seen that beef burgers containing raw potato starch had the lowest levels of total HAs (5.5 ng/g), followed by beef burgers containing FOS D (7.9 ng/g), potato fiber (10.2 ng/g), wheat bran (13.3 ng/g), and methylcellulose (14.7 ng/g). The beef burgers containing pectin, HiMaize, FOS A, and FOS B yielded higher levels of total HAs (24.4−42.5 ng/g) than did the reference beef burger containing only NaCl and TPP (16.2 ng/g). The beef burgers containing FOSs A, B, and C gave the highest amounts of MeIQx, 7.1−24.5 vs 0.1−1.4 ng/g for the other ones. Concerning PhIP, the addition of carbohydrates did not have any further effects, and the amounts were generally similar as with the beef burger containing only NaCl and TPP. Exceptions were potato starch that showed a considerably lower amount, while that of pectin was higher. Concerning Norharman, the addition of carbohydrates generally did not have any further effects as compared with the beef burger containing only NaCl and TPP. Exceptions were pectin, HiMaize, and FOS A that contained higher amounts. As compared with the ordinary beef burgers, the beef burgers containing carbohydrates or NaCl and TPP generally had lower contents of PhIP and Norharman. The average recoveries of HAs during extraction of the beef burgers were around 65, 65, 50, and 45% for MeIQx, 4,8-DiMeIQx, PhIP, and Norharman, respectively.

Table 3. Percent of HAs that Were Bound to the Carbohydrate Fraction When 20 mg of Each Polysaccharide Was Dissolved in 1 mL of Water Together with 100 μL of a Reference Solution Containing 2 ng/μL of 10 Different HAs[a]

	% binding	
HA	potato fiber	wheat bran
MeIQx	35	27
7,8-DiMeIQx	43	15
4,8-DiMeIQx	29	28
PhIP	47	38
Norharman	59	49
Harman	66	55
Trp-P-1	78	69
Trp-P-2	80	70
AαC	42	49
MeAαC	37	43

[a] The mixture was incubated and thereafter centrifuged, and the binding fraction was analyzed by HPLC.

Weight Loss during Cooking. Weight loss during frying varied considerably for the beef burgers (**Table 2**). Depending on the carbohydrate in the beef burger, it ranged from 25% for those containing HiMaize and potato fiber to 45% for those containing FOS B. All beef burgers containing carbohydrates had lower cooking loss than the ordinary beef burger; however, only the beef burgers containing potato fiber or HiMaize had lower cooking loss than the reference beef burger containing only NaCl and TPP. The cooking loss for the ordinary beef burger was 46%, and the cooking loss for the beef burger with only NaCl and TPP was 26%.

Binding Test. Wheat bran and potato fiber were the only carbohydrates that bound to the HAs when a solution of 10 HAs was mixed with water and carbohydrates and then centrifuged (**Table 3**). Binding to wheat bran and potato fiber was most pronounced for Trp-P-1, Trp-P-2, Norharman, and Harman. For the other carbohydrates, no binding was observed.

The binding of HAs to wheat bran and potato fiber was broken during solid phase extraction and did not affect the analysis.

DISCUSSION

The amount of HAs in the beef burgers in the present study is well in accordance with similar studies (*17*, *28*−*30*). In a previous study, we concluded that the weight loss during cooking had a great impact on the formation of HAs (*17*). There, beef burgers with NaCl and TPP, which exhibited small cooking loss, were compared to ones without additives, which exhibited a large weight loss. This high weight loss during cooking resulted in an increased formation of HAs, possibly due to increased transport of precursors to the surface. Therefore, in the present study, we continued to investigate the effect of weight loss on the amount of HAs. To do this, various carbohydrates known to bind water were added to the samples. In addition to the various carbohydrates, NaCl and TPP were added to all beef burgers, except the ordinary one. Although NaCl and TPP may partially mask the effect of the carbohydrates by competing for binding sites, this study was designed to have consumer-accepted beef burgers comparable to commercially beef burgers. Therefore, NaCl and TPP together with common carbohydrates used in meat products were tested in this study. The amounts of TPP used fall into allowed and used ranges (*31*).

Surprisingly, all beef burgers with addition of carbohydrates, except the beef burgers with potato fiber or HiMaize, exhibited higher weight loss than did the reference beef burger containing only NaCl and TPP. Polysaccharides modify and control the mobility of water in food systems. The weight loss during cooking depends on the ability of the carbohydrate to bind water during the preparation and cold storage of the beef burgers and the ability to retain the water during heat treatment. Potato fiber and wheat bran are complex mixtures of fibers and can be regarded as more inert materials, leading to less water binding of the beef burger. The ratio between amylose and amylopectin is 80/20 in HiMaize and 20/80 in potato starch, which might explain the different weight losses (*32*). However, it cannot be excluded that other factors may have an effect. Other studies investigating the effect on water holding by addition of carbohydrates to meat products have shown that results may vary depending on the concentrations of carbohydrates, other interfering additives, and the type of meat product (*13*−*15*, *33*).

The ordinary beef burger had a high weight loss, as compared with the beef burger containing only NaCl and TPP (46 vs 26%), and the amounts of PhIP are 23.9 and 8.2 ng/g raw meat, respectively (**Table 2**). This result is in accordance with results from our previous study (*17*). The effect on HA formation after adding NaCl and TPP in that study seemed to depend on the reduction in weight loss during frying, while in the present study HA formation seems to depend both on the reduction in weight loss and on the type of the carbohydrate added.

The amount of PhIP vs weight loss during frying is shown in **Figure 1**. Only the beef burger containing pectin gave a higher weight loss and higher amounts of PhIP than the reference beef burger, indicating that this type of pectin should not be added to beef burgers. The beef burger with HiMaize gave approximately the same weight loss and amount of PhIP as the reference beef burger, thus there were no benefits from adding HiMaize. In this study, a large group of beef burgers containing wheat bran, potato fiber, FOSs, guar gum, and methylcellulose exhibited various weight loss properties but showed about the same content of PhIP. Thus, from **Figure 1**, it can be concluded that beef burgers with potato fiber give the lowest weight loss and reduce the amount of PhIP.

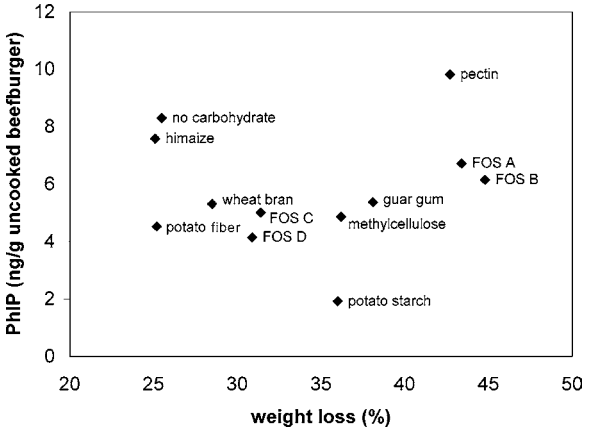

Figure 1. Amount of PhIP in beef burgers containing NaCl, TPP, and various carbohydrates in relation to weight loss during frying.

Interestingly, the addition of some types of carbohydrates (raw potato starch, FOS A, and FOS B) resulted in high weight losses but comparatively low concentrations of PhIP. The formation of PhIP using, e.g., potato starch, was 77% lower as compared with the reference beef burger. This finding is similar to those in a previous study, where the addition of 2.8% potato starch markedly reduced the weight loss in fried beef burgers from 35 to 20% and inhibited the formation of mutagens by 22% (*18*). Because this result might have been due to a chemical effect of the glucose in starch, glucose and lactose were added and the mutagenicity decreased even more. These results suggest that mutagenic formation is inhibited by decreased transport of precursors as well as by increased sugar content. An optimal effect on the formation of HAs in model systems has been reported when mono- or disaccharides are present at about half the molar amount of creatine and free amino acids; however, when the concentration of sugar was increased, the formation of mutagens decreased (*18*, *34*−*36*). Sugars in excess may inhibit HA formation and probably monomers from the carbohydrate chain in starch might have been released and thus reduced the amount of HAs. The beef burger containing the two FOSs, FOS A and FOS B, gave approximately the same result, a high weight loss, but a comparatively low amount of PhIP, as expected from the weight loss; see **Figure 1**. The two other FOSs, FOS C and FOS D, gave a lower weight loss and also a lower concentration of PhIP, which was more as expected from the weight loss. The reasons for the differences may be difficult to speculate upon; all FOSs originated, e.g., from chicory root. However, the fractionation processes may have been different leading to products with various characteristics in terms of water-holding capacity, arrangement of the molecules, and content of other components such as sugars. It has been shown that the addition of 0.5−2.5% FOS to beef burgers reduced the amount of PhIP, MeIQx, and DiMeIQx by 19−58% (*19*). However, no data about the weight loss during cooking for the beef burgers were given in that study. The authors address the inhibiting effect to the reducing sugars coming from decomposition of oligosaccharides and inulin.

For the beef burgers containing FOS A, FOS B, and FOS C, the amounts of MeIQx were higher than in the other beef burgers, including the ordinary one (**Table 2**). Fructose may affect MeIQx differently than PhIP, and it has been shown that inulin can be depolymerized into fructose during heat treatment and participate in the Maillard reaction (*28*). Fructose has been shown to produce more mutagens than glucose when heated together with creatine and glycine (*34*). The carbohydrates added may also participate in the Maillard reaction and form other

products. In contrast to the results from the present study, a decrease in MeIQx formation was observed when frying beef burgers containing FOSs (*19*). The HA formation reaches a maximum at certain monosaccharide levels, while monosaccharides in excess have an inhibiting effect (*18*, *34*, *35*).

Wheat bran and potato fiber were the only carbohydrates in this study that bound to HAs. These two additives differ in structure from the others by being highly insoluble and the only ones consisting of a more complex mixture of polysaccharides but also considerably amounts of protein; see **Table 1**. Furthermore, both wheat bran and potato fiber contain lignin, which is a complex group of aromatic polymers normally present in woody plant tissues. Similarly, previous studies have shown the binding to be reversible, increasing with lignin content of the fiber (*20*) and hydrofobicity of the carcinogen (*37*). The uptake of the HAs in the intestines of rat was reported to be decreased due to binding to insoluble fibers such as wheat bran and sorghum (*38*, *39*). Thus, the addition of wheat bran or potato fiber not only reduces the amount of HAs and gives a low weight loss during cooking of beef burgers but may also reduce the uptake in the intestines. The extraction method of HAs was able to break this bond, and the binding of HAs to wheat bran or potato fiber did not interfere with the extraction recovery.

In conclusion, the addition of NaCl and TPP reduced the formation of HAs in beef burgers. By adding certain carbohydrates (potato starch, potato fiber, wheat bran, and FOS D) at low concentrations to the amounts of HAs can be further decreased. Of the 11 different carbohydrates tested, raw potato starch appeared to be most capable of inhibiting the total formation of HAs. Adding small amounts of carbohydrate is a simple and effective way of reducing the amount of HAs and can easily be applied in households and commercial preparations of beef burgers. In addition, carbohydrates containing lignin such as wheat bran or potato fiber may bind to HAs and decrease the absorption in the intestines.

ABBREVIATIONS USED

AαC, 2-amino-9H-pyrido[2,3-*b*]indole; 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; Harman, 1-methyl-9H-pyrido[3,4-*b*]indole; HAs, heterocyclic amines; HPLC, high-performance liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; MeAαC, 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; Norharman, 9H-pyrido[3,4-*b*]indole; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; TPP, sodium tripolyphosphate; Trp-P-1, 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]-indole; Trp-P-2, 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole.

LITERATURE CITED

(1) Doll, R.; Peto, R. The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer. Inst.* **1981**, *66*.

(2) Augustsson, K.; Steineck, G. Cancer risk based on epidemiological studies. In *Food Borne Carcinogens Heterocyclic Amines*; Nagao, M., Sugimura, T., Eds.; Wiley: West Sussex, England, 2000; pp 332−347.

(3) *Food, Nutrition and the Prevention of Cancer: A Global Perspective*; American Institute for Cancer Research: Washington, DC, 1997.

(4) De Stefani, E.; Deneo-Pellegrini, H.; Mendilaharsu, M.; Ronco, A. Meat intake, heterocyclic amines and risk of colorectal cancer: A case control study in Uruguay. *Int. J. Oncol.* **1997**, *10*, 573−580.

(5) Butler, L. M.; Sinha, R.; Millikan, R. C.; Martin, C. F.; Newman, B.; Gammon, M. D.; Ammerman, A. S.; Sandler, R. S. Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. *Am. J. Epidemiol.* **2003**, *157*, 434−445.

(6) Nowell, S.; Coles, B.; Sinha, R.; MacLeod, S.; Luke Ratnasinghe, D.; Stotts, C.; Kadlubar, F.; Ambrosone, C.; Lang, N. Analysis of total meat intake and exposure to individual heterocyclic amines in a case-control study of colorectal cancer: Contribution of metabolic variation to risk. *Mutat. Res.* **2002**, *506*−*507*, 175.

(7) Sinha, R.; Kulldorff, M.; Chow, W.-H.; Denobile, J.; Rothman, N. Dietary intake of heterocyclic amines, Meat-derived mutagenic activity, and risk of colorectal adenomas. *Cancer Epidemiol. Biomarkers Prev.* **2001**, *10*, 559−562.

(8) Augustsson, K.; Skog, K.; Jägerstad, M.; Dickman, P. W.; Steineck, G. Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney: A population-based study. *Lancet* **1999**, *353*, 703−707.

(9) Sugimura, T. Nutrition and dietary carcinogens. *Carcinogenesis* **2000**, *21*, 387−95.

(10) Pais, P.; Salmon, C. P.; Knize, M. G.; Felton, J. S. Formation of mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats, and meat drippings. *J. Agric. Food Chem.* **1999**, *47*, 1098−108.

(11) Jägerstad, M.; Laser Reutersvärd, A.; Öste, R.; Dahlqvist, A.; Olsson, K.; Grivas, S.; Nyhammar, T. Creatinine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. In *The Maillard Reaction in Foods and Nutrition*; Waller, G., Feather, M., Eds.; ACS Symposium Series 215: Washington, DC, 1983; pp 507−519.

(12) Skog, K. I.; Johansson, M. A.; Jägerstad, M. I. Carcinogenic heterocyclic amines in model systems and cooked foods: A review on formation, occurrence and intake. *Food Chem. Toxicol.* **1998**, *36*, 879−896.

(13) Shand, P. J.; Sofos, J. N.; Schmidt, G. R. Properties of algin/calcium and salt/phosphate structured beef rolls with added gums. *J. Food Sci.* **1993**, *58*, 1224−1230.

(14) Desmond, E. M.; Troy, D. J.; Buckley, D. J. The effects of tapioca starch, oat fibre and whey protein on the physical and sensory properties of low-fat beef burgers. Z. *Lebensm. Wiss. Technol.* **1998**, *31*, 653−657.

(15) Tsai, S.-J.; Unklesbay, N.; Unklesbay, K.; Clarke, A. Water and absorptive properties of restructed beef products with five binders at four isothermal temperatures. *Z. Lebensm. Wiss. Technol.* **1998**, *31*, 78−83.

(16) Troutt, E. S.; Hunt, M. C.; Johnson, D. E.; Claus, J. R.; Kastner, C. L.; Kropf, D. H. Characterisics of low-fat ground beef containing texture-modifying ingredients. *J. Food Sci.* **1992**, *57*, 19−24.

(17) Persson, E.; Sjoholm, I.; Skog, K. Effect of high water-holding capacity on the formation of heterocyclic amines in fried beef burgers. *J. Agric. Food Chem.* **2003**, *51*, 4472−4477.

(18) Skog, K.; Jägerstad, M.; Reuterswärd, A. L. Inhibitory effect of carbohydrates on the formation of mutagens in fried beef patties. *Food Chem. Toxicol.* **1992**, *30*, 681−688.

(19) Shin, H. S.; Park, H.; Park, D. Influence of different oligosaccharides and inulin on heterocyclic aromatic amine formation and overall mutagenicity in fried ground beef patties. *J. Agric. Food Chem.* **2003**, *51*, 6726−6730.

(20) Vikse, R.; Bålsrud Mjelva, B.; Klungsoyr, L. Reversible binding of the cooked food mutagen MeIQx to lignin-enriched preparations from wheat bran. *Food Chem. Toxicol.* **1992**, *30*, 239−246.

(21) Sjödin, P. B.; Nyman, M.; Nilsson, L.; Asp, N.-G.; Jägerstad, M. Binding of 14C-labeled food mutagens (IQ, MeIQ, MeIQx) by dietary fiber *in vitro*. *J. Food Sci.* **1985**, *50*, 1680−1684.

(22) Augustsson, K.; Lindblad, J.; Övervik, E.; Steinbeck, G. A population-based dietary inventory of cooked meat and assessment of the daily intake of food mutagens. *Food Addit. Contam.* **1999**, *16*, 215−225.

(23) Busquets, R. B. M.; Toribio, F.; Puignou, L.; Galceran, M. T. Occurrence of heterocyclic amines in several home-cooked meat dishes of the Spanish diet. *J. Chromatogr. B* **2004**, *802*, 79−86.
(24) Thomson, B. M.; Lake, R. J.; Cressey, P. J.; Knize, M. G. Estimated cancer risk from heterocyclic amines in ccoked meat—A New Zealand perspective. *Proc. Nutr. Soc. N. Z.* **1996**, *21*, 106−115.
(25) Gross, G. A.; Grüter, A.; Heyland, S. Optimization of the sensitivity of High-preformance liquid chromatography in the detection of heterocyclic aromatic amine mutagens. *Food Chem. Toxicol.* **1992**, *30*, 491−498.
(26) Torbio, F.; Puignou, L.; Galceran, M. T. Evaluation of different cleanup procedures for the analysis of heterocyclic aromatic amines in a lyophilized meat extract. *J. Chromatogr. A* **1999**, *836*, 223−233.
(27) Bang, J.; Nukaya, H.; Skog, K. Blue chitin columns for the extraction of heterocyclic amines from cooked meat. *J. Chromatogr. A* **2002**, *977*, 97−105.
(28) Shu, C.-K. Flavor components generated from inulin. *J. Agric. Food Chem.* **1998**, *46*, 1964−1965.
(29) Knize, M. G.; Dolbeare, F. A.; Carroll, K. L.; Moore, D. H.; Felton, J. S. Effect of cooking time and temperature on the heterocyclic amine content of fried beef patties. *Food Chem. Toxicol.* **1994**, *32*, 595−603.
(30) Sinha, R.; Rothman, N.; Salmon, C. P.; Knize, M. G.; Brown, E. D.; Swanson, C. A.; Rhodes, D.; Rossi, S.; Felton, J. S.; Levander, O. A. Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. *Food Chem. Toxicol.* **1998**, *36*, 279−87.
(31) National Food Administration, Sweden, LIVSFS, 2003; p 20.
(32) Henningsson, A. M.; Margareta, E.; Nyman, G. L.; Bjorck, I. M. Influences of dietary adaptation and source of resistant starch on short-chain fatty acids in the hindgut of rats. *Br. J. Nutr.* **2003**, *89*, 319−328.
(33) Mansour, E. H.; Khalil, A. H. Characteristics of low-fat beefburger as influenced by various types of wheat fibers. *Food Res. Int.* **1997**, *30*, 199−205.
(34) Skog, K.; Jägerstad, M. Effect of monosaccharides and disaccharides on the formation of food mutagens in model system. *Mutat. Res.* **1990**, *230*, 263−272.
(35) Shin, H. S.; Strasburg, G. M.; Ustunol, Z. Influence of different unifloral honeys on heterocyclic aromatic amine formation and overall mutagenicity in fried ground-beef patties. *J. Food Sci.* **2003**, *68*, 810−815.
(36) Bordas, M. M., E.; Puignou, L.; Galceran, M. T. Formation and stability of heterocyclic amines in a meat flavour model system—Effect of temperature, time and precursors. *J. Chromatogr. B* **2004**, *802*, 11−17.
(37) Ferguson, L. R.; Roberton, A. M.; Watson, M. E.; Kestell, P.; Harris, P. J. The adsorption of a range of dietary carcinogens by α-cellulose, a model insoluble dietary fiber. *Mutat. Res.* **1993**, *319*, 257−266.
(38) Ferguson, L. R.; Zhu, S.; Kestell, P. Contrasting effects of nonstarch polysaccharide and resistant starch-based diets on the disposition and excretion of the food carcinogen, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), in a rat model. *Food Chem. Toxicol.* **2003**, *41*, 785−792.
(39) Sjödin, P.; Nyman, M.; Nielsen, L. L.; Wallin, H.; Jägerstad, M. Effect of dietary fiber on the disposition on excretion of a food carcinogen (2-^{14}C-Labeled MeIQx) in rats. *Nutr. Cancer* **1992**, *17*, 139−151.
(40) Nyman, M.; Asp, N. G. Fermentation of dietary fibre components in the rat intestinal tract. *Br. J. Nutr.* **1982**, *47*, 357−366.

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