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# Influence of Different Oligosaccharides and Inulin on Heterocyclic Aromatic Amine Formation and Overall Mutagenicity in Fried Ground Beef Patties

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The effects of different oligosaccharides [fructooligosaccharide (FOS), galactooligosaccharide (GOS), and isomaltooligosaccharide (MOS)] and inulin on heterocyclic aromatic amine (HAA) formation and overall mutagenicity in fried ground patties were evaluated. Different oligosaccharides and inulin was added directly to ground beef. Patties (100 g) were fried at 225 °C (surface temperature) for 10 min per side. FOS added at levels of 0.5, 1.0, 1.5, 2.0, and 2.5 g to 100 g of ground beef inhibited total HAA formation by 19, 32, 45, 51, and 58%, respectively. The addition of 1.5 g of FOS, GOS, MOS, and inulin to ground beef patties inhibited total HAA formation by 50, 47, 46, and 54%, respectively. They also reduced overall mutagenicity by 52, 51, 48, and 59%, respectively. These studies confirm that oligosaccharides and inulin have the potential to reduce HAA formation in cooked beef patties.

**KEYWORDS: Heterocyclic aromatic amine; mutagenicity; oligosaccharide; inulin; ground beef**

## INTRODUCTION

Epidemiological studies have shown that diet and lifestyle are closely related to human cancer (*1*). Many mutagens and carcinogens have been identified in foods. Recently, several foods and constituents of foods have been investigated for their inhibitory or promotional effects on carcinogenesis (*2*, *3*).

Heterocyclic aromatic amines (HAAs) are produced in muscle foods during cooking, and many HAAs have been shown to be mutagenic and/or carcinogenic (*1*). These compounds have been classified into two categories: pyrolytic mutagens and thermic mutagens. Pyrolytic mutagens are formed when proteins, amino acids, or proteinaceous foods are heated to high temperatures (>300 °C) and are characterized by a pyridine ring with an amino group attached (*4*, *5*). Thermic mutagens are formed at lower temperatures (<300 °C), and several have been identified in cooked fish and meat products. These compounds, also called aminoimidazoazaarenes, have been characterized as quinolines, quinoxalines, pyridines, or furopyridines. The most commonly found HAAs in foods 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-phenylimidazo[4,5-*b*]pyridine (PhIP) (*3*−*5*).

It has been reported that concentrations of HAAs or overall mutagenicity in fried ground beef patties can be reduced by the addition of compounds such as cherry tissue (*6*), vitamin E (*7*), garlic-related sulfur compounds (*8*, *9*) soy protein concentrate (*10*), defatted glandless cottonseed flour (*11*), unifloral honeys (*12*), and tea polyphenolics (*13*, *14*).

Oligosaccharides are a group of short-chain nondigestible polysaccharides that occur naturally in foods. They are typically defined as glycosides that contain between 3 and 10 sugar moieties and are characterized by the type and sequence of the monosaccharide moieties present (*15*). Initially, oligosaccharides were introduced as sucrose substitutes and for use as bulking agents in foods. Later, it was determined that certain oligosaccharides had the potential to increase intestinal microflora in the colon (*16*−*18*). Thus, because of their prebiotic properties, oligosaccharides have received much recent attention as functional food ingredients (*19*).

Currently, there are nine types of oligosaccharides commercially produced (*15*). Fructooligosaccharides (FOS), galactooligosaccharides (GOS, or transgalactosyloligosaccharides, TOS), and soybean oligosaccharides have been most extensively studied for providing the best evidence of prebiotic effects in humans (*15*, *19*). Inulin, on the other hand, was initially used primarily as a fat substitute in food products (*20*). Inulin is heterogeneous with respect to polymer chain length. Its degree of polymerization (DP) ranges from 3 to 60, but it primarily consists of DP 20−25 (*21*).

It has been demonstrated that the addition of carbohydrates such as glucose and fructose is effective in inhibiting the formation of HAA in cooked beef patties, resulting in the reduction of overall mutagenicity related to a decrease in HAA formation (*22*, *23*). Sugar is viewed as a major contributor to HAA formation, but the addition of sugars to ground beef patties at levels ranging from 2 to 4% reduced HAA formation and the overall mutagenicity of cooked ground meat (*23*). It has been reported that the addition of reducing sugars to meat beyond the optimum needed for formation of HAAs results in the formation of Maillard reaction products, which inhibit the mechanism of HAA formation (*24*). However, there is no information on the effect of oligosaccharides and inulin on the formation of meat carcinogens, particularly HAAs. The objective of this study was to evaluate the potential of oligosaccharides

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and inulin as inhibitors of HAA formation and overall mutagenicity in cooked ground beef patties.

## MATERIALS AND METHODS

**Safety.** All HAAs are mutagenic and/or carcinogenic; accordingly, all extractions, separations, and handling of pure compounds were performed with appropriate safety precautions, including the use of goggles, latex gloves, and efficient fume hoods.

**Materials.** Dimethyl sulfoxide (DMSO) was purchased from Fluka Chemical Co. (Buchs, Switzerland). Fructooligosaccharide (FOS; >41%, glucose and sucrose; >33%) was obtained from CJ Corp. (Seoul, Korea), and galactooligosaccharide (GOS; >50%) and isomaltooligosaccharide (MOS; >58%) were obtained from Corn Products International Inc. (Westchester, IL). Inulin (99%) was obtained from Orafti Active Food Ingredients (Malvern, PA). The HAA standards (MeIQx, 4,8-DiMeIQx, and PhIP) were obtained from Toronto Research Chemicals (Toronto, Canada). The HAA standard [Flavor and Extracts Manufacturer's Association (FEMA)] and the internal standard, caffeine, were gifts from Dr. Mark Knize, Lawrence Livermore National Laboratory (Livermore, CA). The FEMA standard contained 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), MeIQx, 4,8-DiMeIQs, and PhIP, each at 5 ng/$\mu$L. Propanesulfonic acid (PRS) Bond-Elut columns (500 mg) and C18 (100 mg) cartridges were purchased from Varian Inc. (Harbor City, CA). Extrelut-20 columns and Extrelut diatomaceous earth were obtained from E. M. Separations Technology (Gibbstown, NJ). All other chemicals were of analytical grade and were purchased from Fisher Scientific (Fair Lawn, NJ).

Freshly ground beef was obtained from a local supermarket and used within 1 h of purchase or stored at −20 °C until required for frying. The fat content was determined according to the method of Folch and others (*25*).

**Preparation of Ground Beef Patties.** Control ground beef patties (100 g ground beef patties containing 1.5 mL of water) and patties containing different oligosaccharides and inulin added separately 2 h prior to frying were prepared. Different oligosaccharides (FOS, GOS, and MOS) and inulin (1.5 g) or FOS (0.5−2.5 g) were mixed and dissolved in 1.5 mL of water, added directly to 100 g of ground beef, and then mixed in a TongYang Magic mixer (TongYang Magic Corp., Seoul, Korea) for 2 min to achieve a uniform dispersal of ingredients and water carrier. Each patty (100 g) was formed by placing the ground beef in a Petri dish (9 cm diameter × 1.5 cm thickness) to ensure patty uniformity. The study was repeated three times using one batch of ground beef with a fat content of 15.3 ± 0.2%.

**Cooking of Patties.** Patties were fried in a Teflon-coated electric frying pan (Chefline Corp., Seoul, Korea) at 225 °C (surface temperature) for 10 min on each side. The surface temperature of the frying pan and the internal temperature of patties were determined using a surface temperature thermometer and a thermocouple thermometer (Pacific Transducer Corp., Los Angeles, CA). The internal temperature of the patties at the end of frying (20 min) was 84 ± 3 °C. Two patties were fried for each replication, and three replicates were analyzed for each treatment. For each replicate, four subsamples were analyzed (two unspiked for concentration and two spiked for recovery). The cooked meat patties were mixed in a blender to produce a uniform sample and frozen at −4 °C until extraction.

**Extraction of HAAs from Ground Beef Samples.** The HAAs were extracted from the meat samples and purified using solid-phase chromatography following the procedure of Gross and Grüter (*26*). Meat samples were extracted by homogenizing 45 g of cooked meat with 105 g of 1 N NaOH. The homogenate was divided into five equal aliquots. To determine extraction recoveries, two of the aliquots were spiked with 250 ng of each of the HAAs (IQ, MeIQ, MeIQx, DiMeIQx, and PhIP) dissolved in 50 $\mu$L of methanol. Samples were mixed with Extrelut diatomaceous earth (Varian, Inc.) to fill an Extrelut 20 column. All five extractions were made with 40 mL of dichloromethane containing 5% toluene (v/v) using attached Bond Elut PRS extraction columns. One unspiked aliquot from each meat sample was processed for the Ames/*Salmonella* assay as indicated below. For mutagenic activity testing, which does not require further sample cleanup, the remaining aliquot was eluted from the PRS cartridge with 2.0 mL of MeOH−$NH_4OH$, evaporated to dryness, and dissolved in 120 $\mu$L of DMSO. For HAA analyses, the PRS cartridges were washed with 6 mL of 0.1 N HCl and 15 mL of 40% methanol in 0.1 N HCl, followed by 2 mL of water. The HAAs were transferred to Bond Elut C18 cartridges (100 mg) with 20 mL of ammonium acetate buffer (0.5 M, pH 8.0). The cartridges were eluted with 0.8 mL of MeOH/$NH_4OH$ (9:1, v/v). The eluates were evaporated to dryness and dissolved in 50 $\mu$L of methanol containing 5 ng/$\mu$L caffeine as an internal standard.

**HPLC Analyses.** Separation of the HAAs was carried out on a TSK-gel ODS80-TM column (25 cm × 4.6 mm i.d.; Tosoh Haas, Montgomeryville, PA). A precolumn (Supelguard LC-8-DB, Supelco, Bellefonte, PA) was attached between the injector port and column to filter out unwanted compounds, and the cartridge was replaced after ~60 injections. The flow rate of the mobile phase was 1 mL/min. The initial ratio of solvent A (acetonitrile) to solvent B (triethylamine phosphate, 0.01 M, pH 3.2) was 8:92, which increased to 17:83 during the first 10 min. The acetonitrile concentration continued to increase until the ratio was 25:75 (10 min) and then 55:45 (10 min). Over the next 5 min, the solvent A/solvent B ratio was increased to 80:20 to facilitate elution of other compounds. After 35 min, the eluting solvent was returned to its initial ratio (8:92) for 10 min to allow the column to re-equilibrate before the next injection. Samples were analyzed on a Chem Station (Agilent Technologies, Palo Alto, CA) with a diode array detector (model 1100, Agilent Technologies) and a scanning fluorescence detector (model 474, Hewlett-Packard, Avondale, PA).

The identities of the peaks were established by comparing retention times of the peaks with those of the corresponding spiked samples analyzed under the same conditions. Furthermore, UV spectral characteristics of the HPLC peaks in each sample were compared with library spectra acquired from standard HAA solutions. For each experiment, before HPLC separation of the sample extracts, four aliquots (10, 15, 20, and 25 $\mu$L) of two standard mixtures of HAAs (containing 0.5 ng/$\mu$L of each compound), the caffeine internal standard (5 ng/$\mu$L), and HAA standard FEMA (5 ng/$\mu$L) were injected. Linear regression (nanograms of compound against peak area) was performed for individual HAAs in each mixture. A correlation coefficient of ≥0.99 was considered to be acceptable for FEMA internal standards and one of ≥0.97 for the laboratory mixtures of HAAs. Each peak area corresponding to an HAA was corrected with the internal standard regression line and expressed as nanograms per gram of cooked meat. The method of Gross and Grüter (*26*) was then used for determining extraction efficiency and for quantification of HAAs. Each data point consisted of four subsamples, two spiked and two unspiked. The average area of the spiked samples minus the average of the unspiked samples allowed comparison with the regression line for the standard mixture. Each data point was then corrected for its individual extraction efficiency or percent yield. Concentrations of each HAA formed were determined using the average of the two unspiked subsamples. The linear regression slope for FEMA was used to determine the amount of each HAA present in each sample.

***Salmonella* Mutagenicity Assay.** The mutagenic activity of the sample extracts was determined using the standard plate incorporation assay described by Ames and others (*27*) using *Salmonella typhimurium* TA98 (Molecular Toxicology, Inc., Boone, NC). Aroclor-induced rat liver S-9 mixture (Molecular Toxicology, Inc.) was used for metabolic activation. DMSO was used as a negative control (spontaneous revertant colonies), whereas 2-aminoanthracene was used as a positive control for *S. typhimurium* TA98. Negative control (spontaneous revertant colonies) and positive control gave averages of 23 and 850 revertants/$\mu$g, respectively. To determine calculated values of revertants per gram of meat, individual HAA standards (MeIQx, DiMeIQx, and PhIP) were tested under similar conditions. The concentrations of HAAs obtained by HPLC analyses were then multiplied by these values to determine the calculated overall mutagenicity. Mutagenic activity was calculated from the linear portion of the dose−response curve using the method of Moore and Felton (*28*). A minimum of four dose points from duplicate platings was used, and the linear portion of the curves was used to calculate the revertants per gram of cooked beef patties.

**Statistical Analyses.** The results were analyzed by Sigma Stat 2.0 (Jandel Corp., San Rafael, CA). One-way analysis of variance

**Table 1.** Effect of Various Fructooligosaccharide (FOS) Concentrations on the Formation of Heterocyclic Aromatic Amines in Fried Ground Beef Patties

| | HAAs[a] (ng/g) | | | total | inhibition (%) of total HAA |
|---|---|---|---|---|---|
| treatment | MeIQx | DiMeIQx | PhIP | HAAs | formation |
| control | 5.5 ± 0.6a | 2.8 ± 0.4a | 16.8 ± 1.4a | 25.1 | |
| 0.5 g of FOS | 4.7 ± 0.3a | 2.2 ± 0.3a | 13.5 ± 0.8a | 20.4 | 19 |
| 1.0 g of FOS | 3.6 ± 0.3a | 1.7 ± 0.2a | 11.7 ± 0.7a | 17.0 | 32 |
| 1.5 g of FOS | 2.8 ± 0.3b | 1.3 ± 0.3b | 9.8 ± 0.5b | 13.9 | 45 |
| 2.0 g of FOS | 2.4 ± 0.2b | 1.3 ± 0.3b | 8.7 ± 0.4b | 12.4 | 51 |
| 2.5 g of FOS | 2.2 ± 0.2b | 1.1 ± 0.2b | 7.2 ± 0.4b | 10.5 | 58 |

[a] Means with different letters are not significantly different ($p < 0.05$). Comparisons are made only within the same column. Means ± standard deviations; $n = 3$ for all treatments.

**Table 2.** Effect of Different Oligosaccharides and Inulin on the Formation of Heterocyclic Aromatic Amines in Fried Ground Beef Patties

| | HAAs[a] (ng/g) | | | total | inhibition (%) of total HAA |
|---|---|---|---|---|---|
| treatment[b] | MeIQx | DiMeIQx | PhIP | HAAs | formation |
| control | 5.7 ± 0.5a | 3.1 ± 0.3a | 17.4 ± 1.5a | 26.2 | |
| FOS | 2.6 ± 0.3b | 1.4 ± 0.4b | 9.2 ± 0.6b | 13.2 | 50 |
| GOS | 2.8 ± 0.4b | 1.6 ± 0.4b | 9.5 ± 0.7b | 13.9 | 47 |
| MOS | 2.8 ± 0.3b | 1.7 ± 0.3b | 9.7 ± 0.6b | 14.2 | 46 |
| inulin | 2.3 ± 0.4b | 1.2 ± 0.3b | 8.6 ± 0.5b | 12.1 | 54 |

[a] Means with different letters are not significantly different ($p < 0.05$). Comparisons are made only within the same column. Means ± standard deviations; $n = 3$ for all treatments. [b] 1.5 g of oligosaccharides and inulin was added to 100 g ground beef patties for treatments.

(ANOVA) was performed. Appropriate comparisons ($p < 0.05$) were made using the Student−Newman−Keuls test for one-way ANOVA. Calculation of mutagenic activity was made by linear regression analysis of the dose−response curves of revertants per microgram of HAAs or gram of cooked beef patties. Correlations between the measured mutagenicity of the fried patties by the *S. typhimurium* assay and calculated mutagenicity from the concentrations of the HAAs in the fried ground beef including control and treatments that were quantified by HPLC were made by linear regression analysis.

## RESULTS AND DISCUSSION

**Reduction of HAA Formation in Ground Beef Patties by Oligosaccharides and Inulin.** The dominant HAA in fried ground beef patties was PhIP, followed by MeIQx, and DiMeIQx (**Table 1**). IQ and MeIQ are found infrequently in cooked beef, and very small concentrations of IQ and MeIQ compounds in cooked beef are problematic because of difficulties with coelution and peak interference (*29*). Average recoveries of HAAs added to the cooked ground beef patties, with or without added oligosaccharides or inulin, were 81 ± 12, 78 ± 16, and 77 ± 16% for MeIQx, 4,8-DiMeIQx, and PhIP, respectively. These recoveries are comparable to those reported by Balogh et al. (*7*) and Shin et al. (*12*). Salmon et al. (*30*) reported recovery percentages ranging from 35 to 98% for IQ, MeIQx, and DiMeIQx and from 9 to 63% for PhIP, whereas Britt et al. (*6*) reported recovery percentages of 84, 73, and 65% for MeIQx, DiMeIQx, and PhIP, respectively, from five replicates of each treatment analysis.

When FOS was added to 100 g of ground beef at levels of 0.5, 1.0, 1.5, 2.0, and 2.5 g HAA formation (i.e., the sum of the concentrations of MeIQx, DiMeIQx, and PhIP) was inhibited by 19, 32, 45, 51, and 58%, respectively (**Table 1**). Analysis of variance revealed that the addition of FOS at levels >1.5% significantly ($p < 0.05$) reduced the formation of HAAs in fried ground beef patties. However, statistical analysis revealed no significant ($p < 0.05$) difference between the percentages of inhibition achived with 1.5−2.5% FOS (**Table 1**). When 1.5 g of different oligosaccharides (FOS, GOS, and MOS) and inulin was dissolved in 1.5 mL of water and added to 100 g of ground beef before frying, HAA formation was inhibited ($p < 0.05$) (**Table 2**). The addition of FOS, GOS, MOS, and inulin to ground beef patties inhibited total HAA formation by 50, 47, 46, and 54%, respectively. Reductions in PhIP concentrations were 47, 45, 44, and 51%, respectively. Different oligosaccharides and inulin also inhibited MeIQx formation in ground beef patties by 54% (FOS), 51% (GOS), 51% (MOS), and 60% (inulin). However, statistical analysis revealed no significant ($p < 0.05$) difference among the degrees of inhibition on HAA formation in fried beef patties by different oligosaccharides and inulin (**Table 2**). The results presented here indicate the potential use of oligosaccharides and inulin as inhibitors of HAA formation in meats.

Several literature reports allude to HAA reduction achieved with the addition of different carbohydrates such as glucose, fructose, and sucrose. Skog et al. (*31*) demonstrated that when glucose and pure lactose or lactose from milk powder were added to beef patties at concentrations up to 4%, mutagenicity was reduced by 34−76%. Shin et al. (*12*) reported that the addition of buckwheat, clover, and sage honey to ground beef patties inhibited total HAA formation by 55, 52, and 51%, respectively. They concluded that carbohydrate components present in honeys are the primary inhibitory compounds in HAA formation in fried ground beef patties. They also observed that the addition of fructose, glucose, or fructose and glucose together to ground beef before cooking reduced ($p < 0.05$) the total HAA formation, with reductions of 42, 41, and 48%, respectively. Oligosaccharides are a group of short-chain polycarbohydrates consisting of different numbers of monosaccharide moieties and different glycosidic linkages (*32*). It is likely that carbohydrates are the primary inhibitory compounds from oligosaccharides and inulin in HAA formation in fried ground beef patties.

It has been established that carbohydrates have a great impact on the formation of HAAs and also change the relative amounts of the HAAs. It has been speculated that HAA formation occurs through intermediates of the Maillard reaction. Jägerstad et al. (*33*) proposed that pyridines and pyrazines, formed via Maillard reaction, react with an aldehyde to form a quinoline or quinoxaline. Such structures are integral parts of the HAA molecule. Creatine undergoes dehydration and cyclization to form creatinine when heated, which then reacts with an aldehyde to form an IQ- or IQx-type of HAA.

However, when excess amounts of carbohydrates are added to meat or a model system containing HAA precursors, formation of HAAs is reduced. Skog and Jägerstad (*22*, *23*) also reported that when glucose in molar excess was added to the model system containing glucose, phenylalanine, and creatinine, the HAA formation was significantly ($p < 0.05$) decreased. The mechanisms by which carbohydrates inhibit HAA formation have not been fully established. One possible explanation is that sugar itself or, more likely, some Maillard reaction products may combine directly with creatine or creatinine and that such a reaction may be competitive with the reaction that produces HAAs. The Maillard reaction is also known to produce antioxidant products (*34*), which may inhibit HAA formation. Skog and Jägerstad (*22*) have shown that a typical Maillard reaction product, 5-(hydroxymethyl)-2-furfural, inhibits mutagenic activity. The Maillard reaction is facilitated

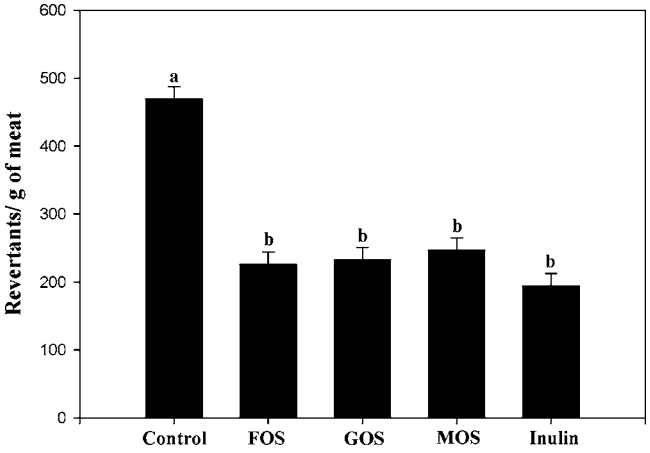


**Figure 1.** Effect of different oligosaccharides and inulin on overall mutagenicity of ground beef patties with *S. typhimurium* TA98. Bars with different letters are significantly different ($p < 0.05$). All treatments were replicated three times. Treatment consisted of the addition of 1.5 g of oligosaccharides and inulin to 100 g ground beef patties.

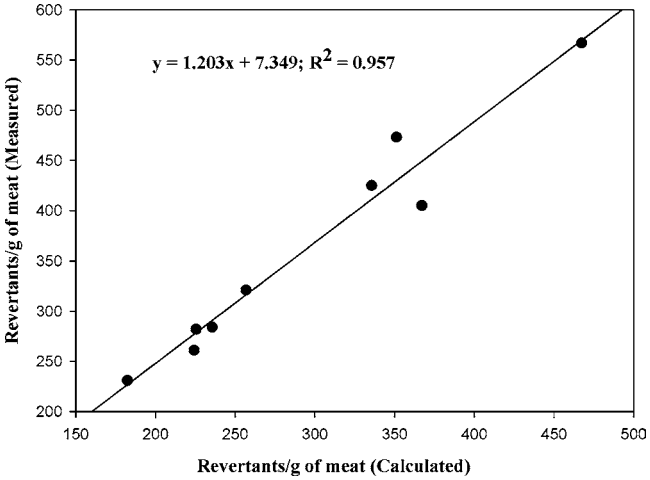


**Figure 2.** Plot of mutagenic activity quantified by *S. typhimurium* TA98 and mutagenic activity calculated from the HAA concentrations in fried beef patties as determined by HPLC analyses. Each point is the mean of three replicated experiments.

by the addition of reducing sugar from the decomposition of oligosaccharides and inulin to the ground beef patties, which contain free amino groups as a meat protein.

Skog and Jägerstad (*22*) also demonstrated that the greatest HAAs could be produced when the amount of glucose was half that of creatin(in)e. Skog and Jägerstad (*22*) demonstrated that a molar ratio between creatine and glucose of 1:1 or 1:2 resulted in a decreased HAA formation in a model system study. Skog et al. (*31*) also reported that the original creatine/glucose molar ratio in a beef sample was about 1:0.4 and that the addition of glucose (1, 2, and 4%) depressed mutagenicity by 34−79%.

**Reduction of Overall Mutagenicity in Ground Beef Patties.** The effects of different oligosaccharides and inulin on the overall mutagenicity of cooked beef patties were evaluated by the Ames *S. typhimurium* assay using the tester strain TA98 (**Figure 1**). FOS, GOS, MOS, and inulin (1.5 g each) significantly ($p < 0.05$) reduced mutagenicity by 52, 51, 48, and 59%, respectively, with the number of revertants being lowered from 467 to 224, 232, 246, and 192 revertants/g of meat, respectively. Some of these results are comparable to those reported by Skog et al. (*31*), who demonstrated a 34−76% reduction in overall mutagenicity in fried beef patties through the addition of glucose or lactose (1−4%). Shin et al. (*12*) showed that overall mutagenicity was reduced by 26−36% when different unifloral honeys (1% buckwheat, clover, or sage honey) were added to ground beef patties before frying. They also demonstrated that the addition of glucose and fructose reduced ($p < 0.05$) mutagenicity, with reductions of 19 and 20% being obtained.

The *S. typhimurium* mutagenicity test is desirable in the evaluation of overall mutagenicity by the reduction of HAA concentrations in meat products with specific potential inhibitors. There is a possibility that other mutagenic compounds may be introduced into the fried ground beef through the interaction of the potential inhibitor with meat components or by the thermal breakdown of the inhibitor itself. The results presented here indicate that the inhibition of HAA formation in fried ground beef patties by oligosaccharides and inulin, as measured by HPLC analyses, is accompanied by a concomitant reduction in the overall mutagenicity of the patties.

The mutagenic activity of each HAA standard was determined by the *S. typhimurium* TA98 assay. The mutagenic activities of MeIQx, DiMeIQx, and PhIP standards determined by the *S. typhimurium* TA98 assay were 82900, 19800, and 1900 revertants/$\mu$g of HAA, respectively. These results agree with the data of Wakabayashi and Sugimura (*5*), who demonstrated that MeIQ is the most mutagenic of the HAAs evaluated, followed by IQ, MeIQx, DiMeIQx, and PhIP. Although PhIP contributes <18% of the total mutagenic activity of meat, it is the most abundant HAA formed in cooked meat (*29*). Therefore, it would be expected that a significant reduction of the mutagenicity of cooked beef patties in this study would also mean that there were meaningful reductions in the concentrations of MeIQx and DiMeIQx in addition to PhIP.

We were interested in correlating the measured mutagenicity of the fried patties by the *S. typhimurium* assay with mutagenicity values calculated from the concentrations of the HAAs in the fried ground beef that were quantified by HPLC. The Ames assay may determine mutagenic activity not totally accounted for by HAA concentrations. The plot of measured and calculated mutagenicity is shown in **Figure 2**. The measured mutagenicity in each sample was quite similar to the mutagenicity value calculated from the determined concentrations of HAAs. These observations agree with those of Felton et al. (*35*), who reported that measured mutagenicity in fried beef patties was similar to the mutagenicity calculated from the measured concentrations of HAAs. The linear regression between measured and calculated activities (slope of 1.20, $R^2 = 0.96$) indicates that the concentrations of the determined HAAs are responsible for most, but not all, of the mutagenicity detected. Other HAAs such as IQ, IQx, MeIQ, 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine (DMIP), and trimethylimidazopyridine (TMIP) are found infrequently in cooked beef, but when present, they are found in very low amounts. Felton and Knize (*36*) reported that PhIP in fried ground beef accounted for 86−91% of the total mass of the mutagenic compounds (*1*).

The results also demonstrated that the addition of different oligosaccharides and inulin did not result in the formation of other mutagenic compounds in the ground beef through the interaction of the potential inhibitor with meat components or by the thermal breakdown of the inhibitor itself. The scatter in the data is probably due to a combination of measurement errors in solid-phase extraction procedures and the accumulation of errors in each analytical method. Shin et al. (*12*) reported that ~80% of the mutagenicity could be accounted for by quantitative HPLC analyses of HAAs in cooked beef and that the *S. typhimurium* mutagenicity assay would be a reasonable screen-

ing method to determine HAA formation in cooked meat samples. On the basis of the results of the present study, the latter procedure could effectively evaluate the effects of selected potential inhibitors on HAA formation in fried ground beef patties.

**Conclusions.** This study demonstrated that the addition of oligosaccharides and inulin may represent an effective approach to reducing HAA formation in cooked beef patties. Reduction of overall mutagenicity was related to the decrease in HAA formation in fried ground beef patties. This study demonstrated that the addition of oligosaccharides and inulin did not result in the formation of other mutagenic compounds in the ground beef through the interaction of the potential inhibitor with meat components or by the thermal breakdown of the inhibitor itself. More research is required to provide a better understanding of mechanisms by which oligosaccharides and inulin inhibit HAA formation.

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