Inhibition of Bacterial Growth by Maillard Reaction Products

Hjörleifur Einarsson,* Benkt Göran Snygg, and Caj Eriksson

Maillard reaction products (MRP) were obtained by refluxing solutions containing either arginine and xylose (AX) or histidine and glucose (HG). The solutions were tested either unfractionated or partly purified by dialysis through a Spectra/Por (R)-6 membrane with a molecular weight cutoff at 1000. The bacteria tested were both pathogenic and spoilage bacteria frequently found in food. The effects of the AX and HG added were assessed on the basis of the growth curves and the MIC (minimum inhibitory concentration) values recorded. The MIC values obtained show that the inhibitory effect of MRP is dependent on the type of MRP and type of bacteria used. As can be seen from the growth curves, the lag phase of the growth was prolonged with increased concentrations of MRP. The results of the dialysis show that the high molecular weight fraction (M_r >1000) was more inhibitory than the low molecular weight fraction (M_r <1000) when tested with *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. The MRP used in this study have in other studies been shown to possess a high antioxidative effect.

The Maillard reaction, or the nonenzymatic browning reaction, is a reaction between amino groups and reducing compounds. In foods the amino compounds are mostly free or protein-bound amino acids and the reducing compounds reducing sugars. Various chemical, physiological, and technological aspects of the reaction have been studied over a long period of time and have recently been reported (Eriksson, 1981).

One aspect has more or less been omitted from systematic studies namely, the influence of Maillard reaction products (MRP) on microorganisms, especially those found in foods, which is surprising since the Maillard reeaction is so common in foods.

Most investigations of the effect of MRP on microorganisms have been done in microbial growth media. These studies show that MRP can stimulate microbial growth (Jemmali, 1969; Field and Lichstein, 1958) or inhibit it (Lewis, 1930; Finkelstein and Lankford, 1957; Mälkki, 1964; Kato and Shibasaki, 1974; Leite et al., 1979). MRP also have an effect on the intestinal microflora of rats (Horikoshi et al., 1981).

There are very few reports on the influence of MRP formed in foods on the food microflora. Wilson and Brown (1953) isolated, from heat-processed fruits and vegetables, substances that were able to inhibit various types of bacteria. Ingram et al. (1955) reported that orange juice that had turned brown is not readily fermented by *Saccharomyces ellipsoideus*.

Besides the effect on microorganisms, some MRP inhibit lipid oxidation (Lingnert and Eriksson, 1981).

The purpose of this study was to investigate the effect of MRP on some pathogenic and spoilage-causing bacteria frequently found in foods, to determine which types or groups of bacteria are affected, and to partially purify the inhibitory substances. The amino acid–sugar mixtures chosen for this study form antioxidative MRP when heated (Lingnert and Eriksson, 1980).

EXPERIMENTAL SECTION

Reagents, Media, and Bacteria. For the synthesis of MRP the following reagents were used: L-arginine monohydrochloride (A) (Fluka AG, Switzerland), L-histidine monohydrochloride monohydrate (H) (Fluka AG, Switzerland), D-glucose (G) (Fischer Scientific Co.), and D-xylose (X) (Merck, Darmstadt, West Germany).

Nutrient broth (NB) and nutrient agar (NA) (OXOID, U.K.) were used for the growth and maintenance of the bacteria. NA and NB were chosen because they are widely used in microbiology; they are also free from sugar that otherwise can form MRP during autoclaving and thus interfere with those added. All bacterial dilutions were made in 0.9% NaCl.

Bacteria with NCTC numbers were obtained from the National Collection of Type Cultures, London, U.K. The *Proteus*, *Salmonella*, and *Streptococcus faecalis* strains were kindly provided by Dr. T. Nilsson (Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden); other bacteria were from SIK's own collection.

Stock cultures of the bacteria were maintained on NA dishes at +4 °C. Every 2 months, the bacteria were grown twice overnight in 45 mL of fresh NB, then streaked on fresh NA dishes, again grown overnight, checked for purity, and then stored at +4 °C.

Prior to the experiments, one colony of the stock culture was transferred to 45 mL of fresh NB and grown once overnight. The overnight cultures were 10-fold diluted to give a starting bacterial concentration of 3000–30 000 bacteria/mL. Liquid cultures were grown in a New Brunswick Controlled Environment Shaker (New Brunswick Co., Inc. NJ) at the desired temperature, shaking at 75 rpm.

Synthesis of MRP. AX-20 was obtained by refluxing 1.0 mol of arginine and 0.50 mol of xylose in 500 mL of deionized water for 20 h. Before the mixture was refluxed the pH was adjusted to 7.0 with potassium hydroxide. After the mixture was refluxed, the pH had dropped to 4.1 and the absorbance at 1:1000 dilution was found to be 0.140 at 450 nm.

AX-5 and HG-5 were obtained by refluxing 0.30 mol of amino acid and 0.15 mol of sugar in 150 mL of deionized water for 5 h. Before the mixture was refluxed, the pH of each solution was adjusted to 7.0 with potassium hydroxide. After the mixture was refluxed, the pH had dropped to 4.4 for AX-5 and 6.3 for HG-5. The absorbance at 450 nm of 1:1000 diluted samples was 0.112 for AX-5 and 0.162 for HG-5. It has been shown (Lingnert and Waller, 1983) that the antioxidative compounds formed in the reaction between histidine and glucose are unstable, especially when diluted and in the presence of air. Therefore, all MRP mixtures were flushed with N_2 and

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kept in tightly closed containers at +4 °C to prevent possible changes in the antimicrobial effect.

Dialysis. Dialysis was performed in Spectra/Por (R)-6 (Spectrum Medical Industries Inc., Los Angeles, CA) membrane tubes with a molecular weight cutoff at 1000; for dialysis heat-sterilized and N_2-flushed water was used.

The retentate, part of the dialysate, and part of the unfractionated material were freeze-dried and stored at +4 °C under N_2. The yield of material with a M_r higher than 1000 was about 5% of the dry weight of the crude reaction mixture. Before use, the unfractionated material and the dialysate were dissolved in sterile water.

Growth Studies. Appropriate volumes of the AX-20 were added to 45 mL of fresh NB in 100-mL Erlenmeyer flasks to give the desired MRP concentration. There was no readjustment of the pH, which fell from about pH 7.5 in the fresh NB medium to about 6.9 after addition of AX-20 to a concentration of 4 mg/mL of NB. Finally, 1 mL of the bacterial dilution was added.

The growth was assayed by estimating the number of bacteria per milliliter. The number of *Salmonella senftenberg* was estimated by 10-fold dilutions of a 1-mL sample. Two 1-mL samples of appropriate dilutions were taken and placed in two 9-cm Petri dishes and mixed with 20 mL of 45 °C NA. The dishes were incubated for 22 h. Then the log of the average number of colonies was calculated.

All other bacteria were counted with a modified spiral plate counting technique (Gilchrist et al., 1977) in the following way. At regular time intervals, 2 mL of the growth medium was withdrawn and placed in a 5-mL sterile, disposable beaker. If necessary, the cultures were diluted to give the approximate concentration of 1000–500 000 bacteria/mL, and 2 mL of that dilution was placed in 5-mL sterile disposable beakers. Approximately 0.03 mL was streaked in duplicate on predried (30 °C for 20–30 h) NA in 9-cm Petri dishes with a spiral plater (Spiral Systems Marketing, Gaithersburg, MD). The volume of NA in the Petri dishes was 15.0 mL.

After 22–24 h of incubation the colonies were counted in a laser bacteria colony counter (LC), Model SODA (Exotech Incorporated, Gaithersburg, MD). The LC was coupled to an ABC-80 computer (Scandia Metric AB, Solna, Sweden) through an interface kindly supplied by K. Remi and P. Adamek, SIK. The computer was preprogrammed with a standard curve equation, and the log of the number of bacteria per milliliter was obtained directly after counting.

Minimum Inhibitory Concentration (MIC). Appropriate volumes of the test substance solutions were added to 9 mL of NB in 1.9 × 15 cm test tubes to give the desired concentrations (0.025, 0.05, 0.1, 0.2, 0.4, 1.0, 2.0, 4.0, and 8.0 mg/mL) after finally adding 0.1 mL of the bacterial suspension. Each concentration was tested in duplicate (Figure 10 and the *Salmonella* in Figure 9) or in quadruplicate (Figure 9 except the *Salmonella*).

The bacteria were grown in the same way as described above. After 22–24 h of incubation, we checked the growth by looking through the test tube against a lit 60-W light bulb.

The MIC value was recorded as the lowest concentration that completely inhibited formation of visible growth.

RESULTS AND DISCUSSION

Growth Studies. The results of the growth experiment are shown in Figures 1–8. Figure 1 shows the growth of *Escherichia coli* NCTC 9001, and Figure 2 shows the growth of *E. coli* NCTC 10418 (commonly used in antibiotic assay and sensitivity testing). *E. coli* found in foods may indicate bad hygienic conditions and improper processing.

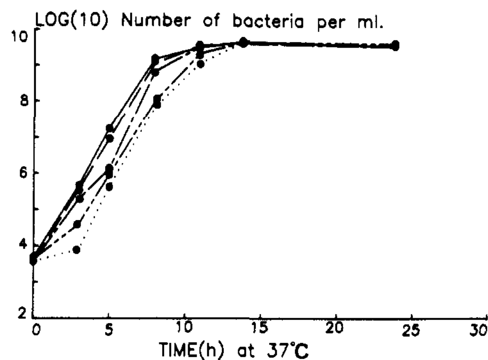

Figure 1. Growth of *E. coli* NCTC 9001 without (—) and with addition of 0.5 (--), 1.0 (---), 2.0 (----), and 4.0 mg/mL (···) of the Maillard reaction mixture AX-20.

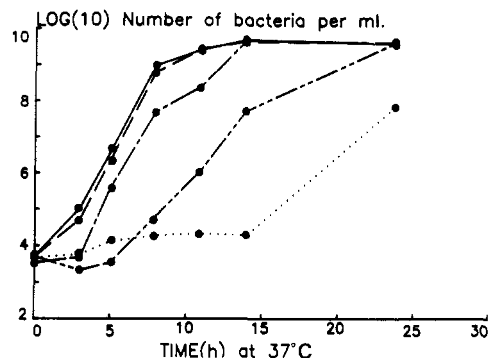

Figure 2. Growth of *E. coli* NCTC 10418 without (—) and with addition of 0.5 (--), 1.0 (---), 2.0 (----), and 4.0 mg/mL (···) of the Maillard reaction mixture AX-20.

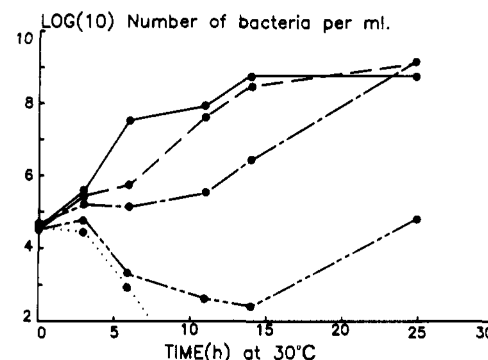

Figure 3. Growth of *Staphylococcus aureus* NCTC 6571 without (—) and with addition of 0.25 (--), 0.5 (---), 1.0 (----), and 2.0 mg/mL (···) of the Maillard reaction mixture AX-20.

The *E. coli* NCTC 9001 was almost totally insensitive. The highest concentration, 4 mg of AX-20/mL, only slightly retarded the onset of the maximum growth rate. *E. coli* NCTC 10418 was more sensitive, and the addition of 4 mg of AX-20/mL prolonged the lag phase to 14 h. *Staphylococcus aureus* is a bacterium associated with food-borne intoxication. Figure 3 presents the growth curves of *S. aureus* NCTC 6571 (commonly used in antibiotic assay and sensitivity testing), and as can be seen, *S. aureus* was more sensitive than the *E. coli* shown in Figures 1 and 2. With the addition of 1 mg of AX-20/mL, there was a pronounced drop in the cell number. This drop could indicate that some bacteria died on exposure to AX, but it could also indicate that some bacteria were injured and not able to grow on the NA. There was no apparent growth after 24 h at 2 mg of AX-20/mL. However, it was observed in a separate test that there were more than one

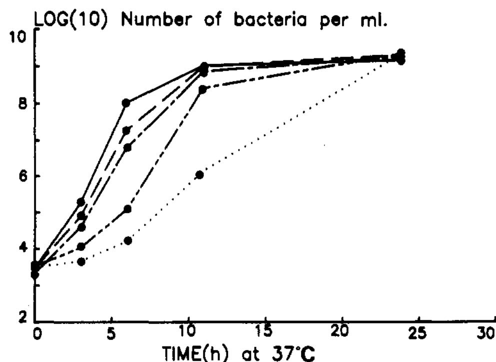

Figure 4. Growth of *Salmonella senftenberg* without (—) and with addition of 0.5 (--), 1.0 (-·-), 2.0 (-··-), and 4.0 mg/mL (···) of Maillard reaction mixture AX-20.

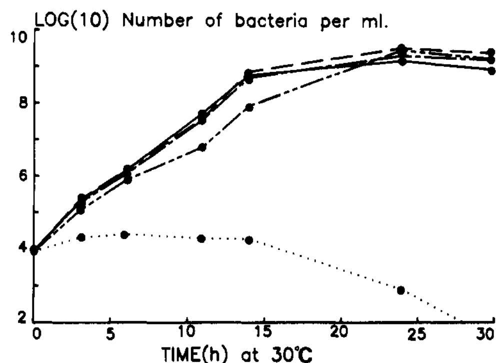

Figure 5. Growth of *Pseudomonas fragi* NCTC 10689 without (—) and with addition of 0.5 (--), 1.0 (-·-), 2.0 (-··-), and 4.0 mg/mL (···) of the Maillard reaction mixture AX-20.

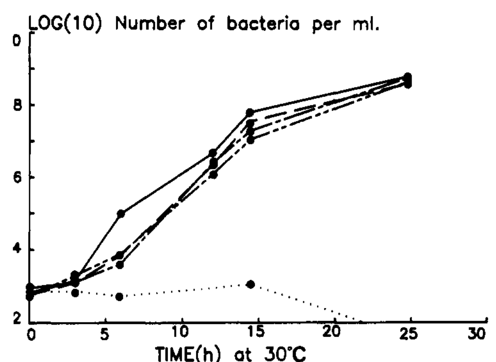

Figure 6. Growth of *Pseudomonas fluorescens* SIK W1 without (—) and with addition of 0.5 (--), 1.0 (-·-), 2.0 (-··-), and 4.0 mg/mL (···) of the Maillard reaction mixture AX-20.

bacteria per mL surviving after 24 h.

In Figure 4 the growth of *Salmonella senftenberg* is shown. *Salmonella* is often associated with meat and egg products, and *Salmonella* exhibited a similar degree of inhibition as the more sensitive *E. coli*.

In Figures 5 and 6 the growth of *Pseudomonas fragi* NCTC 10689 and *Pseudomonas fluorescens* SIK W1, respectively, is shown.

The pseudomonads are bacteria often associated with low-temperature spoilage of foods such as meat and seafood. *P. fluorescens* SIK W1 is also strongly lipolytic (Andersson, 1980). The growth of *Pseudomonas* was apparently not much affected by AX-20 up to 2 mg/mL. At a concentration of 4 mg of AX-20/mL there was no change in the cell number for the first 12–15 h. After that period of time the cell number began to decrease. This phenomenon was not observed with the other bacteria studied.

In Figure 7 the growth of *Lactobacillus plantarum* is shown. *L. plantarum* is found in naturally fermented

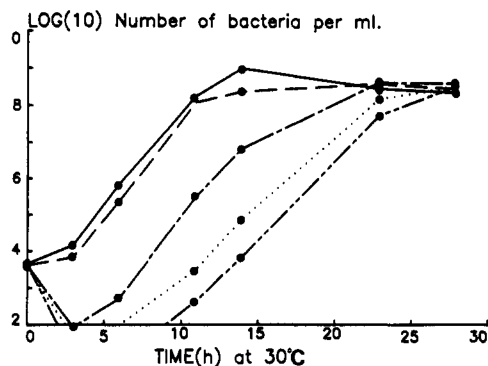

Figure 7. Growth of *Lactobacillus plantarum* without (—) and with addition of 0.06 (--), 0.12 (-·-), 0.25 (-··-), and 0.25 mg/mL (···) of the Maillard reaction mixture AX-20.

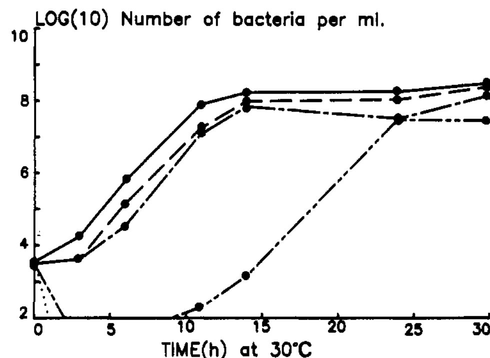

Figure 8. Growth of *Bacillus subtilis* NCTC 3610 without (—) and with addition of 0.06 (--), 0.12 (-·-), 0.25 (-··-), and 0.50 mg/mL (···) of the Maillard reaction mixture AX-20.

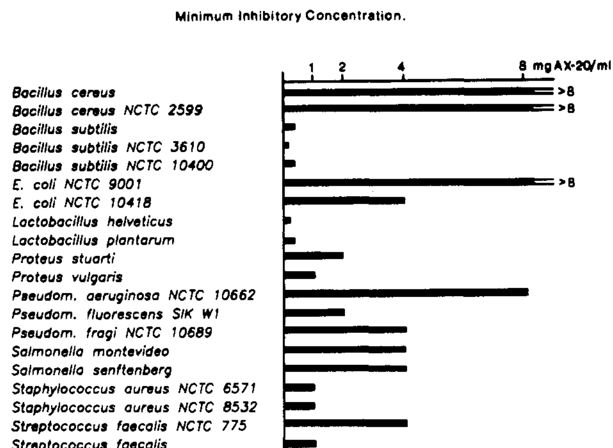

Figure 9. Minimum inhibitory concentration (MIC) of the Maillard reaction mixture AX-20.

foods like sauerkraut. Besides the concentrations shown in Figure 7, two higher concentrations of MRP were used (0.5 and 1.0 mg of AX-20/mL). At these concentrations the number of bacteria was between 1 and 100 per mL from 3 h and throughout the experiment.

Figure 8 shows the growth of *Bacillus subtilis* NCTC 3610. *B. subtilis* was found to be the most sensitive bacterium tested. After 30-h incubation 1-mL samples were taken from the flasks having MRP concentrations of 0.5, 1, and 2 mg of AX-20/mL and inoculated in 45 mL of fresh NB. After 48 h of incubation, growth was observed in samples from the flasks having 0.5 and 1 mg of AX-20/mL but not from the flask having 2 mg of AX-20/mL. This indicates that there was less than one bacteria per mL able to grow after 30 h contact with 2 mg of AX-20/mL.

From the growth curves, the general conclusion can be drawn that it is the lag phase (i.e., the time before the onset

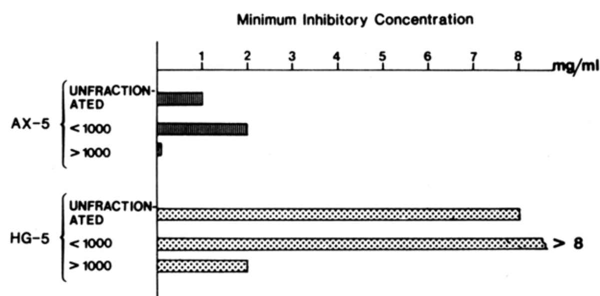

Figure 10. MIC before and after fractionation by dialysis (molecular weight cutoff at 1000) of the Maillard reaction mixtures AX-5 and HG-5 tested with *Bacillus subtilis*.

of the maximum growth rate) that is prolonged by MRP, but the maximum growth rate and final concentration of bacteria are not much affected.

MIC Test. The results from the MIC test are shown in Figures 9 and 10. The results from the replicates gave almost exclusively the same results. The Student's *t* test gave a significant difference between different MIC values. The MIC value varied for different bacteria and even between different strains of the same bacterium. The two *Bacillus cereus* strains tested were found to be insensitive, while the three *B. subtilis* strains were very sensitive.

As in the growth study, the *E. coli* strains showed marked differences in the degree of inhibition by MRP. *E. coli* NCTC 10418 and the two *Salmonella* strains are Gram-negative enterobacteria and had both the same high MIC value, while *Proteus*, which is also a Gram-negative enterobacterium, was found to be more sensitive.

The Gram-positive bacteria, *B. subtilis*, *Lactobacillus*, and *S. aureus*, had MIC values of 1 mg/mL or less.

Good agreement was found between the results from the MIC test and the growth curve experiments. Growth curves obtained by counting the number of bacteria are much more laborious, and only a few variables can be tested at the same time. The color of MRP makes optical methods for obtaining growth curves less versatile. Therefore, the MIC test may be preferred for screening purposes; however, even with this method high concentrations of MRP cannot be used due to interfering color, since it then may be difficult to observe MIC for insensitive bacteria.

Dialysis. The fractionated and unfractionated AX-5 and HG-5 were tested with *B. subtilis*. The results are shown in Figure 10. AX-5 was found to exert a higher inhibitory effect than HG-5. The result also shows that the retentate (i.e., the high molecular weight fraction) had a greater effect than the dialysate (i.e., the low molecular weight fraction).

The MIC value of the AX-5 retentate was found to be only about 0.05 mg/mL as compared with 2.0 mg/mL for the dialysate and 1.0 mg/mL for the unfractionated mixture.

The MIC value of the HG-5 retentate was 1 mg/mL as compared with more than 8 mg/mL for the dialysate and 8 mg/mL for the unfractionated mixture.

The fractionated and unfractionated AX-20 were tested with *B. subtilis*, *E. coli* NCTC 10418, and *S. aureus* NCTC 6571, with the same methods as for AX-5 and HG-5.

The MIC value of the AX-20 was 0.4 mg/mL for the unfractionated mixture, 2 mg/mL for the dialysate, and 0.05 mg/mL for the retenate when tested with *B. subtilis*. For *E. coli* NCTC 10418 the MIC values were 4 mg/mL for the unfractionated mixture, greater than 8 mg/mL for the dialysate and 0.4 mg/mL for the retentate. For *S. aureus* NCTC 6571 the MIC values were 1 mg/mL for the unfractionated mixture, 1 mg/mL for the dialysate, and 0.4 mg/mL for the retentate.

When tested with *B. subtilis* the MIC values of AX-5 and AX-20 were almost the same. The unfractionated AX-20 shows slightly higher effect than AX-5, indicating that more inhibitory compounds were formed during prolonged heating. The fractions showed no such difference. The high molecular weight fraction also had more inhibitory efect on *E. coli* NCTC 10418 and *S. aureus* NCTC 6571 than the dialysate and the crude mixture.

Concerning the mode of action of MRP on bacteria, only general comments can be made. MRP have earlier been shown to possess a number of characteristics that could explain their antibacterial effect. MRP can bind metals (Gomyo and Horikoshi, 1976), proteins can precipitate in the presence of MRP (Horikoshi and Gomyo, 1976) and in the Maillard reaction, and substances like hydroxymethylfurfural (HMF) are formed, which are known to be able to inhibit the growth of bacteria (Ingram et al., 1955). HMF could explain the inhibitory effect of the low molecular weight fraction, but it is evident that the high molecular fraction possesses higher inhibitory effect.

MRP can also interfere with the genetic material and cause mutations (Spingarn and Garvie, 1979).

It is likely that for the unfractionated mixture one or more of these factors are important. For the high molecular weight fractions a more specific mode of action is likely to operate.

This study shows that MRP formed in reaction mixtures of histidine–glucose and arginine–xylose had an inhibitory effect on bacterial growth. It also shows that both the pathogens and spoilage-causing bacteria frequently found in foods were inhibited by MRP. These results are consistent with those of many other investigations, showing that Gram-positive bacteria are generally more sensitive to inhibitory substances than Gram-negative bacteria.

The influence of MRP formed by other amino acids and sugars than those used in the present work, and the influence of different reaction time and pH is under investigation. The effect of growth conditions, e.g., variation of temperature and pH, and the mode of action of MRP will be studied.

LITERATURE CITED

Andersson, R. Ph.D. Thesis, University of Lund and SIK—The Swedish Food Institute, 1980.
Eriksson, C., Ed. *Prog. Food Nutr. Sci.* **1981**, *5* (1–6).
Field, M. F.; Lichstein, H. C. *J. Bacteriol.* **1958**, *76*, 485.
Finkelstein, R. A.; Lankford, C. E. *Appl. Microbiol.* **1957**, *5*, 74.
Gilchrist, J. E.; Donnelly, C. B.; Peeler, J. T.; Campbell, J. E. *J. Assoc. Off. Anal. Chem.* **1977**, *60*, 807.
Gomyo, T.; Horikoshi, M. *Agric. Biol. Chem.* **1976**, *40*, 33.
Horikoshi, M.; Gomyo, T. *Agric. Biol. Chem.* **1976**, *40*, 1531.
Horikoshi, M.; Ohmura, M.; Gomyo, T; Kuwabara, Y.; Ueda, S. *Prog. Food Nutr. Sci.* **1981**, *5*, 223.
Ingram, M.; Mossel, D. A. A.; deLange, P. *Chem. Ind. (London)* **1955**, 63.
Jemmali, M. *J. Appl. Bacteriol.* **1969**, *32*, 151.
Kato, N.; Shibasaki, I. *J. Ferment. Technol.* **1974**, *52*, 177.
Leite, S. R. d. A.; Imasato, H.; Bobbio, P. A. *Rev. Microbiol.* **1979**, *10*, 100.
Lewis, I. M. *J. Bacteriol.* **1930**, *19*, 423.
Lingnert, H.; Eriksson, C. E. *J. Food Process. Preserv.* **1980**, *4*, 161.
Lingnert, H.; Eriksson, C. E. *Prog. Food Nutr. Sci.* **1981**, *5*, 453.
Lingnert, H.; Waller, G. R. *J. Agric. Food Chem.* **1983**, *31*, 27.
Mälkki, Y. In "Microbial Inhibitors in Food"; Molin, N.; Erichsen, A., Eds.; Almqvist and Wiksell: Stockholm, 1964; p 347.
Spingarn, N. E.; Garvie, C. T. *J. Agric. Food Chem.* **1979**, *27*, 1319.
Wilson, D. C.; Brown, H. D. *Food Technol. (Chicago)* **1953**, *7*, 250.

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Trace Analysis of Cinnamaldehyde in Animal Feed, Human Urine, and Wastewater by Electron Capture Gas Chromatography

William M. Blakemore* and Harold C. Thompson, Jr.

Cinnamaldehyde is a popular food flavoring agent that is under toxicological evaluation at the National Center for Toxicological Research. Methods that ensure purity, concentration, homogeneity, and stability of the chemical in feed and the safety of personnel and the environment were prerequisites for the toxicological tests. The chemical is extracted from feed with a solution of 40% methanol, 5% acetic acid, and 55% deionized H_2O. For levels >10 ppm, the feed extract is partitioned against benzene and assayed directly by FID-GC. For feed levels <10 ppm, the benzene extract is derivatized with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBH) for analysis by EC-GC. Wastewater and human urine are extracted with benzene and derivatized. Wastewater is assayed by EC-GC while extracts of human urine require additional cleanup on a silica gel column prior to EC-GC analysis. Theoretical minimum detactable levels of cinnamaldehyde in feed, human urine, and wastewater were 1 ppm, 5 ppb, and 1 ppb, respectively.

The principal ingredient of cinnamon oil is cinnamaldehyde (CNMA) (formula shown in eq 1), which is also present in cassia oil. It is a popular food flavoring and is used as a component of cosmetics, soaps, and detergents. Its use as a flavor and fragrance ingredient was estimated to be 5.00×10^8 g/year, based upon sales in the United States as reported by the U.S. International Trade Commission (1978). Its use as a fragrance ingredient was estimated by Opdyke (1979) to be 4.5×10^7 g/year. CNMA is used as a flavoring ingredient in fruits and juices at levels as high as 6400 ppm, 3500 ppm in baked goods, 2200 ppm in breakfast cereals, 2000 ppm in baby food and desserts, and 1100 ppm in chewing gum.

Jenner et al. (1964) reported an oral LD_{50} for CNMA in rats of 2.22 g/kg, while Sporn et al. (1965) reported 3.35 g/kg and Zaitsev and Rakhmanina (1974) reported 3.4 g/kg. Jenner et al. (1964) explained that rats were characterized by depression, diarrhea, and scrawny appearance and deaths occurred within 2–3 h after administration of cinnamaldehyde. Zaitsev and Rakhmanina reported oral LD_{50}s of 3.4 g/kg for guinea pigs and mice. They noted no differences in LD_{50}s for different sexes of rats, guinea pigs, or mice.

Current human toxicity data for CNMA is limited to its irritant and sensitizing properties. It is a primary irritant that has been implicated as a frequent cause of allergic reactions to perfumes by Schorr (1975). Epidemiological studies or case reports have not been found in the literature that link CNMA exposure to human cancer. However, there are at least two related compounds that have been reported to induce tumors in experimental animals. Schoental and Gibbard (1972) reported that 3,4,5-trimethoxycinnamaldehyde was found to induce tumors in rats after intraperitoneal injection. A report from the National Cancer Institute (NCI) (NCI/NTP, 1980), for a study performed under the National Toxicology Program (NTP), states that cinnamyl anthranilate induced tumors in both rats and mice when administered in the diet at 15 000 or 30 000 ppm.

CNMA is oxidized to cinnamic acid and excreted in the urine as benzoic or hippuric acid. Teuchy et al. (1971) reported that as much as 25% of a dose may be excreted in the urine as hippuric acid within 24 h after an intraperitoneal injection of the compound.

While there is currently no evidence for the carcinogenicity of CNMA, this compound is a potential alkylating agent that could react with cellular macromolecules. This potential, together with human exposure to the compound at relatively high concentrations, and the demonstration of tumorigenic activity of two related compounds, makes the chemical a prime candidate for carcinogenic bioassay. These studies were proposed to be conducted at the National Center for Toxicological Research (NCTR). Prerequisites for these studies were development of chemical methodology to ensure that accurate doses of CNMA were administered to the test animals via spiked feed and that the chemical was uniformly distributed and stable during preparation of the feed as well as during the feeding period of the animal study. Preparation of a stable homogeneous dosed diet required selection of proper medium by which this volatile compound could be added to the feed. Methods are required for the trace analysis of all compounds tested at the NCTR, in the urine of laboratory personnel to monitor their exposure to the compounds and in wastewater to ensure that none of the compounds are discharged into the environment.

Heikki et al. (1972) reported preparation of 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds for subsequent analysis by electron capture gas chromatography (EC-GC). Koshy et al. (1975), and Nambara et al. (1975) described methods for derivatizing carbonyl groups with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBH) in pyridine for EC-GC analysis. Levine et al. (1981) described O-alkyloxime derivatization of aldehydes for gas chromatographic–mass spectrometric analysis.

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