

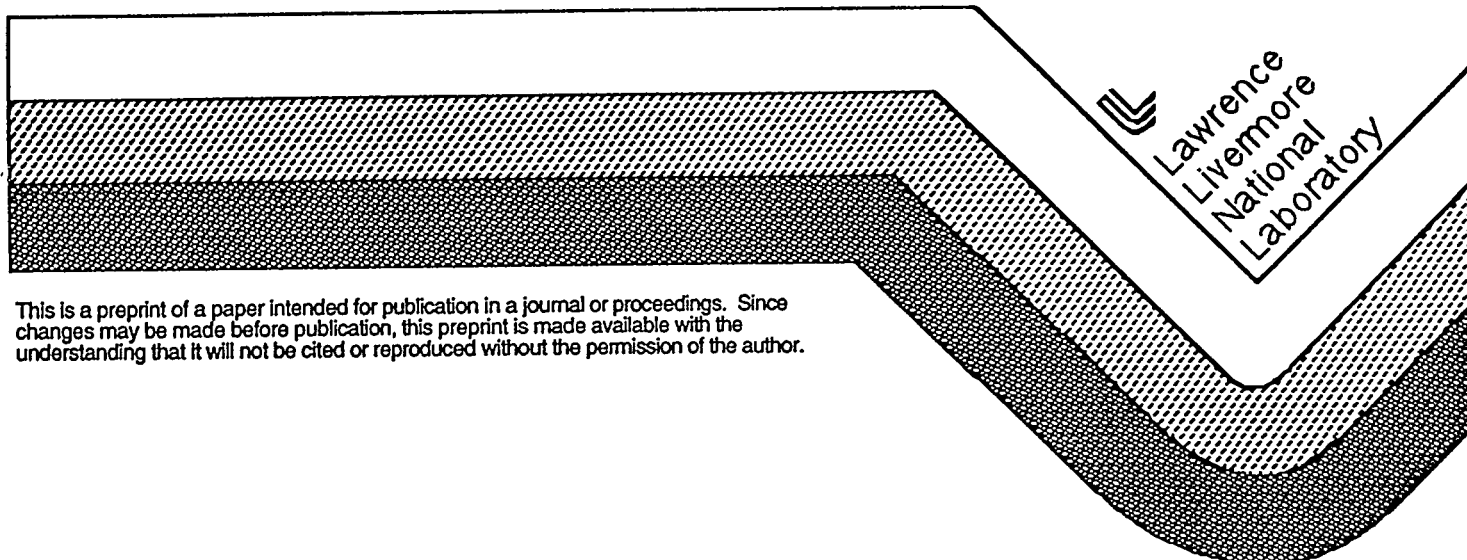
Conf-9409260--1

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This paper was prepared for submittal to the
Euro-Food Tox IV Meeting, Poland, September 12 - 16, 1994

June 1994



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CHARACTERIZATION OF MUTAGENIC ACTIVITY IN GRAIN-BASED
COFFEE-SUBSTITUTE BLENDS AND INSTANT COFFEES

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Summary

Several grain-based coffee-substitute blends and instant coffees showed a mutagenic response in the Ames/*Salmonella* test using TA98, YG1024 and YG1029 with metabolic activation. The beverage powders contained 150 to 500 TA98 and 1150 to 4050 YG1024 revertant colonies/gram, respectively. The mutagenic activity in the beverage powders was shown to be stable to heat and the products varied in resistance to acid nitrite treatment. Characterization of the mutagenic activity, using HPLC and the Ames test of the collected fractions, showed the coffee-substitutes and instant coffees contain several mutagenic compounds, which are most likely aromatic amines.

Introduction

The main food mutagens found in cooked meat and fish products are heterocyclic aromatic amines, these being either amino acid pyrolysates or aminoimidazo[1,2-a]pyridines (1), and are formed from creatine, amino acids and sugars at normal cooking temperatures (2). All heterocyclic amines identified in cooked meats are potent bacterial mutagens and all heterocyclic amines tested have been shown to be carcinogenic in several animal species, including monkeys (3,4). A relationship between consumption of fried meat products and an elevated risk of colon and other cancers has been shown in several epidemiological studies (5,6,7,8). Some non-meat foods, such as toasted breads and cookies

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.

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(9,10), and individual food components such as flour and gluten (11,12), also showed mutagenic activity. Recently, the mutagenic activity in cooked, grain-food products was suggested to be due to the presence of unidentified heterocyclic amines (13). Mutagenic activity in coffee has been reported in *Salmonella* strain TA100 without metabolic activation due to the presence of methylglyoxal (14). Interestingly, mutagenic activity in *Salmonella* strain TA98 with metabolic activation has been reported in two studies (15,16). We investigated the mutagenic activity in instant hot beverage powders; six grain-based coffee-substitute blends and two instant coffees. We quantified the mutagenic response using sensitive *Salmonella* strains and characterized the mutagenic compounds by chromatographic comparison with the heterocyclic amines formed during cooking of muscle meats.

Materials and methods

Six grain-based coffee-substitute blends and two instant coffees were examined. The coffee substitute blends were made from ingredients like malt, barley, chicory and rye. The beverage powders were extracted using the solid-phase extraction method of Gross (17). The extracted samples were tested with *Salmonella* strain TA98, YG1024 or YG1029 with metabolic activation (18). Fractions were collected from HPLC of the extracts, to produce mutagenic activity profiles. The nitrite-resistance of the purified samples was tested using the method of Tsuda et al. (19).

Results

The mutagenic activity in the coffee-substitute blends and instant coffees, measured in *Salmonella* strain TA98, YG1024 and YG1029 with metabolic activation, is shown in Table 1. A higher response in strain YG1024 for all products indicates that the mutagenic compounds are metabolized with O-acetyltransferase (20).

Table 1: Mutagenic activity of coffee-substitute blends and instant coffees.

Sample	TA98 rev/gE*	YG1024 rev/gE*	YG1029 rev/gE*
#1 coffee substitute	310	4050	1130
#2 coffee substitute	380	2660	1520
#3 coffee substitute	240	3340	2450
#4 coffee substitute	340	2100	1980
#5 coffee substitute	280	2650	2800
#6 coffee substitute	150	1150	1240
#7 instant coffee	240	2730	440
#8 instant coffee	500	3290	n.d.

*Mutagenic activity with metabolic activation from the linear portion of dose-response curves from replicate platings. n.d.=not detectable, a non-significant slope from dose-response curves.

Figure 1 shows the mutagenic activity profiles for a coffee-substitute and an instant coffee sample.

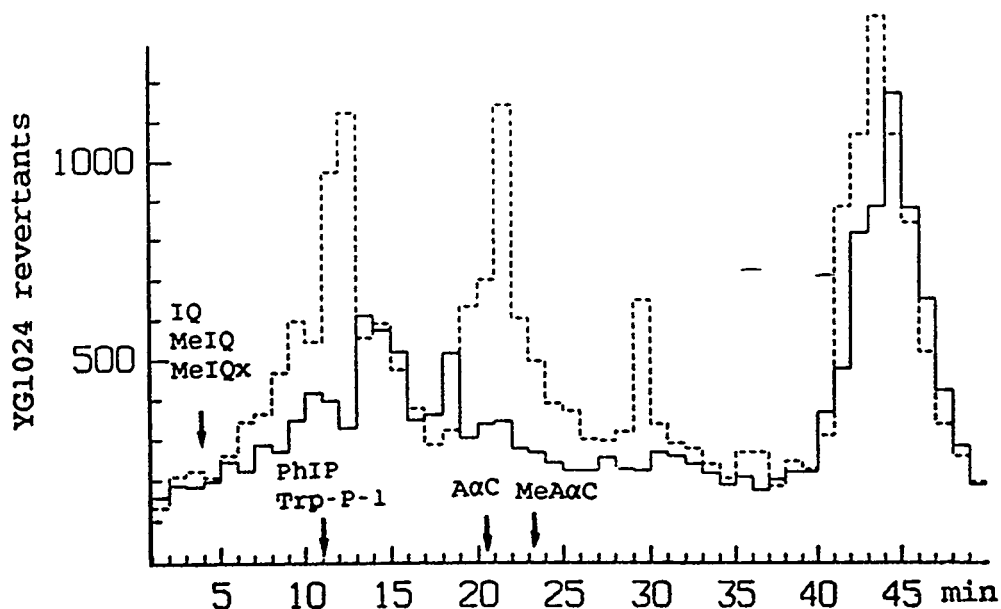


Figure 1: Mutagenic activity profiles for a coffee-substitute (—) and an instant coffee sample (.....).

Each sample has several mutagenic fractions which elute at similar retention times as those of known heterocyclic amines found in the pyrolysis products of foods. All of the products, appear to contain 3 to 4 mutagenic compounds. The retention times of heterocyclic amine standards are also

shown in the figure. The nitrite-resistance test showed the mutagenic activity in coffee-substitute blend #4 and instant coffee #8 to be resistant to acid nitrite treatment. However, the mutagenic activity in coffee-substitute blend #5 was sensitive to acid nitrite treatment.

Discussion

The bacterial responses show that the extracts cause primarily frame-shift mutations, suggesting that aromatic amine or nitro compounds are present (20). The increased response observed using *Salmonella* strain YG1024 as compared to TA98 indicates that the compounds need O-acetyltransferase for their metabolism. The fact that metabolic activation is required suggests that the mutagenic response is due to amines, which require further enzymatic conversion to generate mutagenic activity. Moreover, their extraction behavior from a basic aqueous solution (suspended on the diatomaceous earth support) further suggests that these mutagens are amines. In analogy with muscle foods, plant seeds contain carbohydrates and free amino acids. Moreover, plant seeds do contain several guanidino compounds (15) of which arginine is the most abundant, and these might be mutagen precursors. The observed mutagenic activity in the coffee and coffee-substitute products are probably produced during the roasting of coffee beans and grains at high temperatures. Coffee substitutes are based mainly on wheat or malted barley with the addition of other plant materials such as chicory, beet root or figs.

For aromatic amines there is a good correlation between mutagenic potency in the Ames/*Salmonella* test and carcinogenic potency in rodents (21). The level of mutagenic activity measured in these beverage powders appears to be generally lower than that in cooked meats, but a rigorous evaluation of the significance of the mutagenic activity requires identification and quantification of the mutagenic compounds. From the mutagenic activity alone it appears that

risk to human health from these foods would probably be much lower than from the intake of heterocyclic amines from cooked meats, but for certain populations with a high consumption of coffee substitutes and/or instant coffee, the risk may be significant.

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*This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.