# Attempt to Decrease the Mutagenicity of Smoked-and-dried Bonito (Katsuobushi) by Boiling Bonito Meat in Green Tea Extract

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(Received March 5, 1999; Accepted March 31. 1999)

**To decrease the mutagenicity of smoked-and-dried bonito (katsuobushi) by boiling bonito meat in green tea extract was attempted. A piece (500 g) of bonito meat was boiled in 2.5 and 5% (w/v) green tea extract, then smoked-and-dried in the usual manner of katsuobushi processing. The mutagenicity of katsuobushi was only slightly decreased by boiling the bonito meat in 5% green tea extract. There is a limit to the use of green tea extract for the preparation of katsuobushi with the aim of decreasing mutagenicity.**

**Key words** —— smoked-and-dried bonito (katsuobushi), green tea extract, mutagenicity

## INTRODUCTION

Smoked-and-dried bonito (katsuobushi in Japanese), a popular seasoning in Japan, contains imidazoquinoxaline-type mutagens, *i.e.*, 2-amino-3,8-dimethyl-imidazo[4,5-*f*]quinoxaline (MeIQx)[1,2] and 2-amino-3,4,8-trimethylimidazo[4,5-*f*] quinoxaline (4,8-DiMeIQx).[3,4] Commercial katsuobushi is produced from raw bonito meat by boiling it in water, smoking-and-drying by wood smoke, removal of tar on the surface and finally molding. The mutagens originate during the heating-and-drying process.[4,5] The Japanese ingest, on average, 1 g of katsuobushi product per person per day, which corresponds to 2 ng of MeIQx.[2] The mutagens in katsuobushi are considered to be probable human carcinogens.[6]

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It is important to decrease the mutagenicity of katsuobushi. Previous studies have shown that boiling raw bonito meat in a solution of epigallocatechin gallate or green tea extract decreases the mutagenicity generated during the subsequent heating-and-drying under experimental conditions.[7] Scavenging of the free radical Maillard intermediates by the phenolic antioxidants, including epigallocatechin gallate and green tea extract, is responsible for prevention of the mutagen formation.[7] In this study, decreasing the mutagenicity of katsuobushi by boiling bonito meat in green tea extract under regular processing conditions was attempted.

## MATERIALS AND METHODS

**Preparation of Katsuobushi Sample** —— The entire factory processing procedure of katsuobushi from raw bonito meat consists of four steps: (1) boiling the meat in water (shajuku), (2) smoking-and-drying (baikan) it by wood smoke, (3) removal of tar on the surface (kezuri), and finally (4) molding (kabitsuke). The manufacturer who provided the test samples for this experiment modified the first step, boiling the bonito meat in green tea extract, then proceeded o the second step in the usual manner. The third and fourth steps were omitted.

Bonito fish (about 3 kg weight) captured in the same southern fishing field was used. Commercially available green tea, 150 g (for 2.5% (w/v) green tea extract) or 300 g (for 5% (w/v) green tea extract), was extracted with 6 liters of boiling tap water for 5 min, and the mixture was passed through a sieve (20 mesh) to remove the solid material. A final eluate concentration of 6 liters was made by the addition of tap water. The lumps of raw bonito meat, each weighing about 500 g, were introduced into boiling tap water (control), 2.5% green tea extract or 5% green tea extract, and each mixture was boiled for 60 min. In order to avoid the concentration of green tea extract, boiling tap water was added intermittently to maintain the original volume.

**Extraction of Mutagen from Katsuobushi** —— Each katsuobushi product (10 g) was finely divided into small pieces and extracted with 200 ml of boiling water for 5 min, then the solid material was filtered off by use of glass-wool that had been washed with diluted hydrochloric acid and water. The mutagens in the solution were extracted by the blue rayon method 8) using blue rayon obtained from Funakoshi (Tokyo,

Japan). Thus, blue rayon (0.2 g) was added to the solution and the solution was shaken at room temperature for 1 h. The blue rayon was recovered. New blue rayon (0.2 g) was added to the solution, and the solution was worked up similarly. Two lots of recovered blue rayon were combined, washed with water, and shaken in 100 ml of methanol–concentrated ammonium hydroxide (1000 : 1, v/v) at room temperature for 30 min. Blue rayon was again shaken in 100 ml of the methanol–ammonium hydroxide solution. The extracts were combined and evaporated to dryness under reduced pressure. The residue was dissolved into 1.0 ml of dimethyl sulfoxide for the mutagenicity assay.

**Mutagenicity Assay** —— Mutagenicity was assayed according to the preincubation method of Yahagi *et al.*[9] using the *Salmonella typhimurium* TA 98 strain[10] with S9 mix. The microsomal S9 system prepared from liver microsomes of a rat treated with phenobarbital and 5,6-benzoflavone and cofactor-1 were obtained from Oriental Yeast Company (Tokyo, Japan). A 0.1 ml aliquot of the dimethyl sulfoxide solution of the test sample (1 g equivalent) was introduced into a plate. Duplicate plates were used for each assay. The number of $His^+$ revertant colonies/plate was obtained after subtracting the background number of spontaneously formed $His^+$ revertant colonies (24—32) /plate.

## RESULTS AND DISCUSSION

Imidazoquinoxaline-type heterocyclic amines (MeIQx and 4,8-DiMeIQx) detected in katsuobushi are mutagenic to *Salmonella typhimurium* TA98 with metabolic activation.[1-4] The mutagens are produced during the smoking-and-drying (baikan) of bonito meat.[4,5] The effect of boiling raw bonito meat in green tea extract before the usual smoking-and-drying process on the mutagen formation in katsuobushi was investigated. Regular sized pieces of bonito meat (about 500 g) were used. Preparations of katsuobushi processed by boiling bonito meat in water, 2.5% (w/v) green tea or 5% (w/v) green tea extract were subjected to the mutagenicity test after purification of the mutagens with blue rayon. The mutagenicity was assayed on *Salmonella typhimurium* TA98 strain with metabolic activation (Fig. 1). The number of $His^+$ revertant colonies/g equivalent of control katsuobushi was estimated to be about 530. The number was not

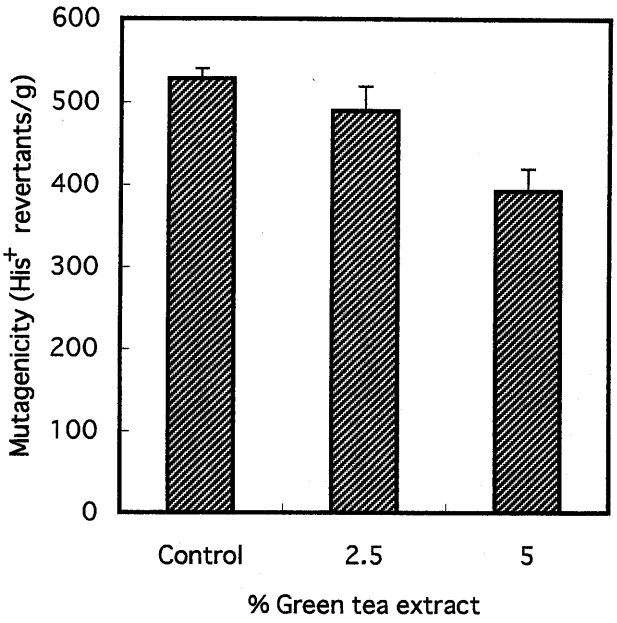


**Fig. 1.** Mutagenicity of Katsuobushi Prepared by Boiling Raw Bonito Meat in Green Tea Extract

A 500 g piece of raw bonito meat was boiled in water (control), 2.5% (w/v) green tea or 5% (w/v) green tea, and subsequently smoked-and-dried in the usual manner of processing katsuobushi. Katsuobushi product was extracted with boiling water, and the extracted mutagens were purified by blue rayon. Mutagenicity was assayed on *Salmonella typhimurium* TA98 with S9 mix. Mutagenicity ± S.D. as expressed by $His^+$ revertant colonies/g equivalent/plate is the mean value of six experiments.

significantly decreased when katsuobushi was prepared by boiling bonito meat in 2.5% (w/v) green tea extract, and the number was only slightly decreased, to 390, when prepared by boiling in 5% (w/v) green tea extract. The concentration of green tea extract at 5% (w/v) may be maximal, because the use of a higher concentration of green tea affected the intrinsic characteristic flavor of katsuobushi.

It has been shown that when much smaller pieces (20 g) of raw bonito meat are boiled in green tea extract at the concentration of 5% (w/v), followed by heating at 100—120℃ for 24—48 h under the experimental conditions, the mutagenicity of the product was greatly decreased, to about 30% of the control.[7] However, the use of the higher concentration of green tea extract in the usual processing of katsuobushi may not have any merit with respect to the flavor of katsuobushi. Hence, there is a limit to the use of green tea extract for the preparation of katsuobushi with decreased mutagenicity.

It has been found that the pyrazine cation radicals generated in the Maillard reaction of sugars/amino acids may be responsible for the

formation of the imidazoquinoxaline-type mutagens.[7,11] Phenolic antioxidants, including epigallocatechin gallate and green tea extract, can scavenge the pyrazine cation radicals and thus inhibit the mutagen formation in model systems.[7,11] However, a large quantity of phenolic antioxidants is required for effective scavenging of the radicals. Food factors other than phenolic antioxidants with stronger scavenger activity against the radicals may be useful for decreasing the mutagenicity of katsuobushi.

**Acknowledgment** The present authors thank Yanagiya Honten in Shizuoka Prefecture for preparing katsuobushi samples.

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