NUTRITIONAL AND TOXICOLOGICAL ASPECTS OF FOOD SAFETY

Edited by

Mendel Friedman

NUTRITIONAL AND TOXICOLOGICAL ASPECTS OF FOOD SAFETY

Edited by

Mendel Friedman

U.S. Department of Agriculture
Agricultural Research Service
Western Regional Research Center
Berkeley, California

PLENUM PRESS • NEW YORK AND LONDON

PREFACE

Naturally occurring antinutrients and food toxicants, and those formed during food processing, adversely affect the nutritional quality and safety of foods. Because of the need to improve food quality and safety by plant breeding, fortification with appropriate nutrients, and processing methods, and because of the growing concern about possible direct relationships between diet and diseases, research is needed to:

(1) evaluate the nutritive quality and safety of crops and fortified, supplemented, and processed foods;

(2) define conditions that favor or minimize the formation of nutritionally antagonistic and toxic compounds in foods; and

(3) define the toxicology, metabolism, and mechanisms of the action of food ingredients and their metabolites.

As scientists interested in improving the safety of the food supply, we are challenged to respond to the general need for exploring:

(1) possible adverse consequences of antinutrients and food toxicants; and

(2) factors which contribute to the formation and inactivation of undesirable compounds in foods.

Medical research offers an excellent analogy. Studies on causes and mechanisms of disease processes are nearly always accompanied by parallel studies on preventive measures and cures. Such an approach offers the greatest possible benefits to the public.

Although much work is needed to define the basic mechanisms of toxic action of food ingredients, enough is known to permit rational research approaches to develop new ways to minimize

effects of antinutrients and toxicants in foods. Such approaches include:

(1) inactivating deleterious compounds with site-specific reagents to prevent them from interacting with living cells;

(2) eliminating the responsible food ingredients from our diet;

(3) breeding new plant varieties which are both nutritious and safe to consume; and

(4) identifying dietary constituents that protect against the adverse action of antinutrients and food toxicants.

For example, fiber in the diet appears to reduce the incidence of cancer. This effect could arise because fiber strongly binds certain carcinogens and food toxicants, thus minimizing or preventing their absorption by the intestine into the blood stream. Since other dietary components affect absorption and excretion of deleterious compounds in foods, dietary protein, carbohydrate, fat, mineral, vitamin, or polyphenol content, as well as fiber, probably may influence the biological action of nutrients, antinutrients, and toxicants in foods. The paper by H. F. Stich and M. P. Rosen on antimutagenic and anticarcinogenic effects of naturally occurring phenolic compounds and that by T. K. Smith and M. S. Carson on the effect of diet on toxicosis of trichothecenes deserve special mention for pointing to promising future research directions on beneficial effects of diet on food safety.

The most important function of a symposium, I believe, is dissemination of insights and cross-fertilization of ideas that will catalyze progress by permitting synergistic interaction among related disciplines. Therefore, in organizing the symposium FOOD SAFETY: METABOLISM AND NUTRITION, sponsored by the Pacific Conference on Chemistry and Spectroscopy, San Francisco, California, October 27-29, 1982, I invited both reviews and reports of recent work. In addition, a number of scientists who did not take part in the symposium accepted my invitation to contribute papers to this volume. This book is, therefore, a hybrid between symposium proceedings and a collection of invited contributions.

I am particularly grateful to Glen A. Bailey of the Pacific Conference for inviting me to organize the symposium, and to all contributors for a well-realized meeting of minds and for excellent collaboration.

The papers are being published under the title NUTRITIONAL AND TOXICOLOGICAL ASPECTS OF FOOD SAFETY as a volume in the series

Advances in Experimental Medicine and Biology. This book is intended to complement the following published volumes in the same series: *Protein-Metal Interactions (1974); Protein Crosslinking: Biochemical and Molecular Aspects (1977); Protein Crosslinking: Nutritional and Medical Consequences (1977); and Nutritional Improvement of Food and Feed Proteins (1978).*

I very much hope that these volumes will be a valuable record and resource for further progress in animal and human nutrition, food safety and toxicology, medicine, and protein chemistry.

Mendel Friedman
Moraga, California
January, 1984

The eyes of all look to you expectantly,
and you give them their food when it is due.
You give it openhandedly, feeding every
creature to its heart's content.

Psalm 145: 15-16

Eat nothing that will prevent you from eating.

Ibn Tibbon, c. 1190

CONTENTS

1

NATURALLY OCCURRING PHENOLICS AS ANTIMUTAGENIC AND ANTICARCINOGENIC AGENTS

Hans F. Stich and Miriam P. Rosin

Environmental Carcinogenesis Unit
British Columbia Cancer Research Centre
Vancouver, B.C., Canada

INTRODUCTION

Epidemiological evidence points to an inverse relationship between the consumption of vegetables and the incidence of cancer at various sites (Hirayama, 1979, 1981; Graham et al., 1978; Mettlin et al., 1981). The search for the protective components in these vegetables has focused on β-carotene and vitamin A (e.g., Bjelke, 1975; Shekelle et al., 1981; Cambien et al., 1980; Peto et al., 1981; Doll and Peto, 1981; Marshall et al., 1982) and ascorbic acid (e.g., Haenszel and Correa, 1975; Kolonel et al., 1981). However, the inverse relationship observed between the ingestion of green/yellow vegetables and the incidence of human cancers could conceivably be due to many other plant components. At present, the percentage contribution of vitamins to the cancer-protective activity of vegetables or fruits is unknown. In this paper, we present results suggesting an involvement of naturally occurring phenolics in the prevention of genotoxicity and carcinogenicity. Since the number of phenolics in various plants is staggering and the discussion of their beneficial or toxic effects is beyond the scope of any short review, we have focused on non-flavonoid simple phenolics (C6), phenolic acids (C6-C1), cinnamic acid and related compounds (C6-C3).

PHENOLICS AS INHIBITORS OF DIRECT-ACTING AND S9-REQUIRING MUTAGENS

Recently, many *in vitro* test systems have been successfully applied to estimate the mutagenic, clastogenic and recombinogenic properties of manmade and naturally occurring chemicals. These rapid and economic bioassays can also be used to uncover the anti-genotoxic and by implication anticarcinogenic activity of compounds

(e.g., Rosin, 1982; Rosin and Stich, 1978a, 1979). The chemical to be examined can be added concurrently, prior to, or after exposure of the test organism to the carcinogen. Results of such studies may help to reveal the mechanism involved in antimutagenic and anticarcinogenic activities of a compound and the better design of treatment protocols. Three examples involving phenolics may exemplify this approach.

Inhibition of Aflatoxin B_1 (AFB_1)-Induced Mutagenesis by Suppression of its Metabolism

AFB_1 and many of its metabolites require activation by the microsomal mixed function oxidase system into a mutagenic (Garner et al., 1972; Wong and Hsieh, 1976) or clastogenic (Stich and Laishes, 1975) species. The ultimate reactive molecule is believed to be AFB_1-2,3-oxide (Lin et al., 1978; Neal and Colley, 1978). The addition of caffeic acid, chlorogenic acid or gallic acid to the S9 activation mixture strongly reduced the mutagenicity of AFB_1 (Fig. 1). Phenolics could exert an inhibitory effect either by suppressing the metabolism of AFB_1 or by trapping the AFB_1-2,3-oxide. The experimental design to find an answer to this question consisted of tracing the formation of AFB_1 metabolites by high pressure liquid chromatography (HPLC) following the addition of phenolics to a mixture containing S9 and AFB_1. The results of this study suggest that the above-mentioned phenolics suppressed the mutagenicity of AFB_1 by interfering with its metabolic activation (Chan, 1982). However, in addition to this action, phenolics could trap the reactive AFB_1-2,3-oxide if it should indeed be formed in their presence. The present study does not exclude this possibility.

Inhibition of Benzo(a)Pyrene Diol Epoxide-Induced Mutagenesis Through Direct Interaction with Phenolics (Wood et al., 1982)

The mutagenicity of the direct-acting benzo(a)pyrene-7,8-diol-9,10-epoxide-2 in *Salmonella typhimurium* and cultured Chinese hamster V79 cells was strongly reduced following the addition of ferulic, caffeic, chlorogenic and ellagic acids. This inhibition of the mutagenicity of the reactive species of a polycyclic aromatic hydrocarbon is assumed to be due to its direct interaction with the phenolics resulting in the formation of complexes.

Inhibition of the Direct-Acting Carcinogen and Mutagen, N-Methyl-N'-Nitro-N-Nitrosoguanidine (MNNG)

Mutagenicity of MNNG was only reduced when phenolics were administered to *S. typhimurium* concurrently with the mutagen (Fig. 2). The tested phenolics (gallic acid, caffeic acid, chlorogenic acid and a commercial preparation of tannins) had no detectable inhibitory effects when added to Salmonella cultures prior to or after their exposure to MNNG (Table 1). It appears likely that

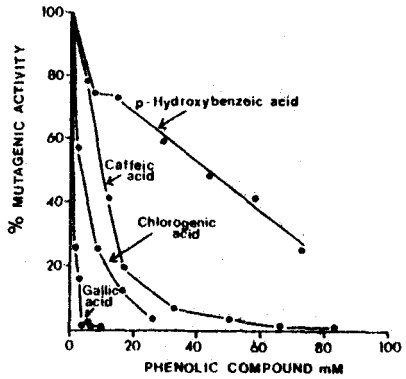

Fig. 1. Effect of several simple phenolics on the mutagenic activity of AFB_1 (3×10^{-5}M) in logarithmically growing *S. typhimurium* TA98 cultures. Mutation frequency of AFB_1 alone was 64 his^+ revertants per 10^7 survivors.

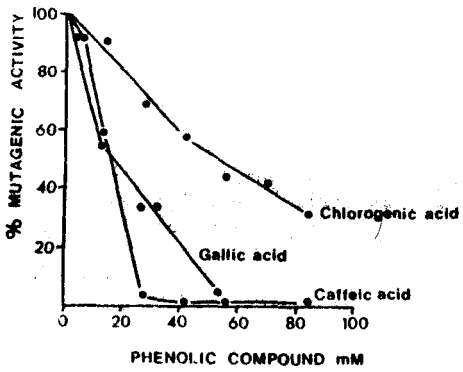

Fig. 2. Effect of chlorogenic acid, gallic acid and caffeic acid on the mutagenic activity of *S. typhimurium* TA1535 cultures exposed to MNNG (3×10^{-5}M). Mutation frequency of MNNG alone was 138 his^+ revertants per 10^7 survivors. Values plotted are percentage of this mutagenic activity which remains when treatments are carried out in the presence of phenolic at concentration indicated. All treatments were non-toxic.

Table 1. Effect of Timing on Inhibition by Chlorogenic Acid of MNNG-Induced Mutagenesis in Salmonella Cultures[a] (Chan, 1982)

	Mutagenic Activity (His^+ Revertants/10^7 Survivors) When Chlorogenic Acid (20 mg/ml) Applied		
No Chlorogenic Acid	Prior to MNNG (3×10^{-5}M)	Concurrent to MNNG (3×10^{-5}M)	After MNNG (3×10^{-5}M)
183	175	69	188

[a] *S. typhimurium* TA1535 logarithmically growing cultures; control frequency, 1 revertant/10^7 survivors. All treatments performed in suspension.

the inhibitory effect is due to a scavenging action by the phenolics of one of the electrophilic decomposition products of MNNG.

PHENOLICS AS INHIBITORS OF MUTAGENICITY RESULTING FROM NITROSATION REACTIONS

Nitrosation of methylurea leads to the formation of direct-acting mutagens which can be readily detected by the *S. typhimurium* mutagenicity assay. This well-defined system was used as a model to examine the effect of several common plant phenolics on nitrosation reactions (Stich et al., 1982a). Gallic acid, caffeic acid, chlorogenic acid, catechin and tannic acid reduced the formation of mutagenic compounds (Table 2). The inhibitory effect of these plant phenolics was similar to or even greater than that of ascorbic acid, which has been widely used to prevent nitrosamine formation *in vitro* and *in vivo* (reviewed by Newmark and Mergens, 1981). The simplest explanation of the inhibitory action of the phenolics is to assume that the yield of mutagenic nitrosation products of methylurea is reduced due to the trapping of the nitrosating species. It is also conceivable that phenolics could interact with nitrosation products and in this way inhibit their mutagenicity (Rosin and Stich, 1978b). However, at the examined doses of phenolics, this mechanism for the inhibitory action appears unlikely, since the phenolics did not significantly reduce mutagenicity when they were added after completion of the nitrosation of methylurea. The mutagenicity results are in general agreement with chemical studies showing an inhibition of N-nitroso compound formation by tannins (Bogovski et al., 1972; Gray and Dugan, 1975), gallic acid (Pignatelli et al., 1976) and several other phenolics (Gray and Dugan, 1975; Groenen, 1977; Kawabata et al., 1979).

Table 2. Inhibition by Phenolics of Bacterial[a] Mutagenicity Due to Nitrosation Products of Methylurea (Stich et al., 1982a)

Phenolics	Concentration	
	Active Range[b]	50% Inhibition
Catechol	0.3-1.0 mM	1.0 mM
Gallic acid	0.7-6.9 mM	2.1 mM
Chlorogenic acid	0.6-6.4 mM	2.1 mM
Tannic acid	0.1-0.9 mg/ml	0.3 mg/ml
Catechin	0.2-3.5 mM	1.0 mM
Resorcinol	0.3-6.4 mM	2.1 mM
p-Hydroxybenzoic acid	No effect	-

[a] *S. typhimurium* TA1535 logarithmically growing cultures were exposed to nitrosation products for 20 min before plating for mutagenicity and toxicity. Phenolics were present throughout the nitrosation reaction. Mutagenicity of nitrosation products formed in the absence of phenolics was 205 his^+ revertants/10^7 survivors.

[b] The range given is the concentration of phenolics in the nitrosation reaction mixture which results in 10-100% inhibition of mutagenicity.

PHENOLIC-CONTAINING FOOD ITEMS AS INHIBITORS OF MUTAGENICITY IN *IN VITRO* TEST SYSTEMS

Human populations are exposed daily to thousands of chemicals in the form of complex mixtures. Their potential or actual health hazard is unknown due to the difficulties of extrapolating *in vitro* results to human populations. Moreover, reliable mutagenicity and carcinogenicity data are usually only available on single pure compounds. Thus the behaviour of a mixture, which is not a simple product of the activation of mutagenic (carcinogenic) and antimutagenic (anticarcinogenic) activities, cannot be predicted from data on single chemicals. Complex interactions between chemicals can lead to "additive", "synergistic", "potentiating" and "antagonistic" effects. The number of combinations and permutations among the hundreds of chemicals which are present in even the simplest food products is so large that an analysis of all the possible interactions just cannot be accomplished. A way out of this dilemma is to simulate *in vitro* the conditions prevailing in man or to examine the genotoxic effects directly in human beings.

The following attempts were made to gain at least some insight into the possible role of phenolic-containing food items as antigenotoxic agents.

1. Three North-American coffee samples were tested for their

capacity to modify the development of mutagenic activity resulting from the nitrosation of methylurea. All three examined instant coffees (one was a decaffeinated brand) suppressed the induction of mutations at and even below concentrations which are customarily used in the preparation of these coffees (Stich et al., 1982a). A similar inhibitory effect was observed when three different teas, including a Japanese, Chinese and Indian brand, were added to the nitrosation mixture consisting of nitrite and methylurea (Table 3). The examined teas and coffees did not reduce the mutagenicity when added after the completion of the nitrosation reaction at concentrations which exerted a strong inhibitory effect when present during nitrosation. Such observations seem to be of particular importance when protocols for epidemiological studies are designed. It is becoming apparent that the sequence of intake of various food items strongly influences the mutagenic and carcinogenic hazard. A simply protective effect of trapping and scavenging food ingredients can only manifest itself when they can intermingle with the reactive forms of mutagens and carcinogens. Only rarely can one find data on this issue, the importance of which seems to be underestimated in epidemiological surveys.

Table 3. Inhibition by Phenolic-Containing Products of Bacterial[a] Mutagenicity Due to Nitrosation Products of Methylurea

Phenolic-Containing Product		Concentration	
		Active Range[b]	50% Inhibition
Coffee:	Instant	0.1-18 mg/ml	1.8 mg/ml
	Instant, decaffeinated	0.1-59 mg/ml	2.6 mg/ml
	Roasted, ground	0.1-60 mg/ml	2.7 mg/ml
Tea:	Japanese	3.2-33 mg/ml	10 mg/ml
	Indian	3.6-102 mg/ml	21 mg/ml
	Chinese	0.8-37 mg/ml	5.3 mg/ml
	Mate	0.6-36 mg/ml	4.7 mg/ml
	Chinese green	0.7-33 mg/ml	4.9 mg/ml
Wine:	White (5 types)	7-510 µl/ml	60 µl/ml
	Red (5 types)	9-350 µl/ml	55 µl/ml
Beer:	4 brands	6-220 µl/ml	34 µl/ml
Betel nut extracts:	Tannin fraction	0.1-1 mg/ml	0.3 mg/ml
	Catechin fraction	0.1-2 mg/ml	0.3 mg/ml
	Flavonoid fraction	0.1-1 mg/ml	0.2 mg/ml

[a,b] See Table 2 for description.

2. Our second attempt to simulate *in vitro* conditions which actually occur during the consumption of a meal dealt with Chinese salt-preserved fish. This particular fish preparation was chosen because of its large consumption in virtually all Asiatic countries, its mutagenic activity following nitrosation (Marquardt et al., 1977), its carcinogenic effect in rodents (Weisburger et al., 1980) and its possible involvement in human cancer (Ho et al., 1978; Huang et al., 1978). The test system consisted of nitrosating (pH 2.0, 1 hr, 37°C) an aqueous fraction of a salt-preserved Chinese fish (Pak Wik) and estimating the frequency of his^+ revertants per survivor of *S. typhimurium* (strain TA1535). Several dietary phenolics and three teas were added to the nitrosation mixture (Fig. 3). Catechin, chlorogenic acid, gallic acid and pyrogallol suppressed the formation of mutagenic nitrosation products. The efficiency of inhibition was comparable to that of ascorbic acid. A Japanese, Chinese and Ceylon tea also prevented the formation of mutagenic nitrosated fish products at doses which are usually consumed by man. Such studies on food products which are actually ingested may, despite the difficulties in handling "messy" mixtures, bridge the gap between epidemiological evidence pointing to a link between consumption of this fish and an elevated risk for gastric cancer and biochemical studies on nitrosation reactions.

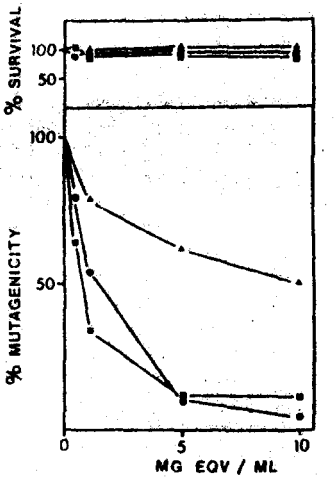

Fig. 3. Inhibitory effect of catechin (■), chlorogenic acid (●) and Chinese tea (▲) on mutagenicity of the nitrite-treated salted fish extract.

ANTICARCINOGENIC ACTIVITY OF PHENOLICS

Several derivatives of cinnamic acid have been reported to inhibit chemical carcinogenesis when present in the diet of treated animals. *p*-Hydroxycinnamic acid, *o*-hydroxycinammic acid, caffeic acid and ferulic acid each caused an inhibition of benzo(a)pyrene-induced neoplasia of the forestomach in female ICR/Ha mice (Wattenberg, 1979; Wattenberg et al., 1980). These phenolics were present from 8 days prior to exposure of the mice to benzo(a)pyrene by oral intubation and continued to be added to the food until 3 days after the last dose of the carcinogen. A reduction was observed in both the percentage of mice with tumours of the forestomach and the number of such tumours per mouse among the cinnamic acid-fed mice. *p*-Hydroxycinnamic acid has also been reported to suppress the development of tumours in the forestomach of ICR/Ha mice receiving the direct-acting carcinogen β-propiolactone (Wattenberg, 1979).

There is a great need for extending these studies in order to obtain a broader understanding of the overall modulating action of naturally occurring phenolics. The specificity of these phenolics for particular carcinogens is unknown. Two structurally related synthetic phenolics, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been shown to be very versatile inhibitors. Their presence leads to a suppression of the activity of a range of carcinogens, including polycyclic aromatic hydrocarbons, diethylnitrosamine, urethane, uracil mustard, 4-nitroquinoline-1-oxide, N-2-fluorenylacetamide, *p*-dimethylaminoazobenzene, methylazoxymethanol acetate, and *trans*-4-amino-3-[2-(5-nitro-2-furyl)-vinyl]-1,2,4-oxadiazole (Wattenberg, 1976, 1979). However, the inhibitory capacity of phenolic compounds is very dependent upon chemical structure, with substituted groups on the phenolic ring affecting the potency of the compound (Wattenberg et al., 1980). Another unresolved issue is the ability of naturally occurring phenolics to affect carcinogen activity at sites removed from the digestive tract. Once again, previous studies have shown that BHT and BHA can suppress dimethylbenz(a)anthracene-induced neoplasms of the lung in mice and of the breast in rats (Wattenberg, 1972, 1973). The effect of the exposure sequence of phenolics and carcinogens to the overall frequency of carcinogenesis in exposed animals is yet another area requiring consideration. Careful studies are now underway for BHA and BHT (King et al., 1983), and should be extended to naturally occurring phenolics.

PHENOLICS AS NITRITE TRAPPERS

Phenolics are known to react with nitrite to form C-nitroso phenolic compounds (Mirvish, 1981; Walker et al., 1982) (Fig. 4). The strength of this reaction depends on the number of hydroxy groups and their position. A fast way to estimate the nitrite