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South Indian Foods: Contaminants and Their Effects

S. Narender Sivaswamy,* B. Balachandran, and S. Balanehru

Isotope Division, Cancer Institute, Adyar, Madras-600 020, India

Life style including dietary habits is one of the most important factors responsible for different types of cancer (Doll and Peto 1981). The role of diet in human cancer has prompted many to analyse the food items, particularly the heat processed foods and food components for possible mutagens and carcinogens (Sato et al. 1986, Shioya et al. 1987, Yano et al. 1988). Food dishes, probably the most complex of all, undergoing various changes during storage, processing and cooking, often form genotoxic compounds (Fishbein 1979). Cooking of proteinaceous food stuffs leads to the formation of amino acid pyrolysates and quinoline compounds which are generally mutagenic in bacterial assays and carcinogenic in experimental animals (Sugimura 1986). Polycyclic aromatic hydrocarbons (PAHs) are formed during combustion, pyrolysis and pyrosynthesis of organic matter (IARC 1983). PAHs have also been identified and quantified in several food items, which proved to be mutagenic and are related to the predominant form of alimentary tract cancers of Asian and Western countries (Sugimura 1986). Epidemiological studies have unequivocally established a relationship between the occurrence of PAHs and different types of cancers. Since the incidence of stomach cancer in South India is very high (about 20% according to our Tumour Registry), we have screened several commonly consumed food dishes and food components for possible contaminants, such as PAHs. These food items were subjected to Ames assay to detect the mutagens (Balachandran et al. 1990). They were assayed for their genotoxic effects, such as chromosomal aberrations, production of micronuclei and sperm head abnormalities. Since many of the Indian food items are stored for long periods, mycotoxin contamination is possible and therefore, we have screened some of the food components for the presence of zearalenone, a *Fusarium* mycotoxin. This paper reports the presence of polycyclic aromatic hydrocarbons (PAHs) and zearalenone in the commonly consumed food items. The mutagenic and genotoxic effects of these food items are also reported.

MATERIALS AND METHODS

The food dishes and food components were procured from the local market in edible form. Some of them were fried in oil (Palm oil, which is generally used in South India)

* Author for all correspondencce and reprint requests.

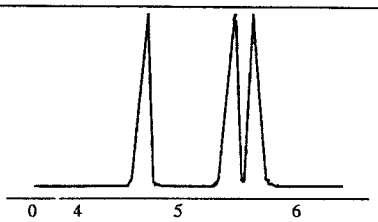

Fig. 1. Chromatogram of authentic samples of PAHs
(Samples procured from Aldrich Co, USA)
a. Chrysene, RT 4.8, Recovery 89.75%
b. Dibenzanthracene, RT 5.6, Recovery 90.3%
c. Benzo(a)pyrene, RT 5.8, Recovery 92.07%

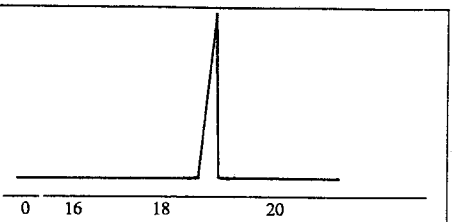

Fig. 2. Chromatogram of authentic sample of Zearalenone
(Sample procured from Aldrich Co, USA)
RT 18.45, Recovery 87.9%

to make them edible and comparable to 'home preparation'. Test sample (listed in Table 1), 500 mg, was ground well with 10 ml of distilled water and extracted three times with 40 ml of dichloromethane. The extracts were pooled and sodium sulphate, 5 g was added to remove excess water and lipids. The clear supernatant was decanted and evaporated over a water bath at 60 C to 5 ml. Cyclohexane, 40 ml was added to it, which was again evaporated to a minimal volume. This was passed through a silica gel (activated at 110 C for 5 h) column of 10 x 50 mm size and eluted with 10 ml of cyclohexane. Sodium sulphate, 1 g was added to the resulting solution. The supernatant was collected and evaporated at 60 C to dryness and the residue was dissolved in 0.5 ml of acetonitrile and used for HPLC analysis (Sivaswamy et al. 1990). The extracted sample, 10 µl was chromatographed in a Shimadzu LC-6A HPLC system (steel column, 150 x 4.6 mm, Zorbaks-ODS; column temperature 35 C; mobile phase: 100% acetonitrile, flow rate, 1 ml/min; 254 nm) and PAHs were detected and quantified based on the chromatograms of authentic samples (Fig. 1).

Food components were screened for the presence of zearalenone (Balanehru et al. 1990). Sample (listed in Table 2), 2.5 g (25ml, in case of liquids) was shaken well with 200 ml of water- ethanol-chloroform (2:50:75, v/v) for 1 h. This was filtered using glass wool and Whatman No.5 filter paper. Two ml of 0.1 M phosphate buffer (pH 7.8) was added and the solution was evaporated at 40 C on a rotary shaker to 2-3 ml. Ten ml of 10% 2-propanol in ether was added to it and was centrifuged for 2 min at 425 x g. The upper etheral layer was separated and chilled for 10-15 min. Two ml of chilled NaOH (0.184 N) was added, vortexed for 11 min and again chilled. This was centrifuged and the upper layer discarded. The lower layer was washed with 2 ml of benzene and chilled before centrifuging to discard the benzene layer. This was neutralised to pH 7.0-7.5 using phenol red indicator and was extracted three times with 2 ml of benzene, after chilling. The pooled extract was centrifuged at 1100 x g for 2 min to seperate the benzene layer, which was later evaporated at 40 C to dryness. The residue was taken in 0.5 ml of water-methanol-acetonitrile (5:3:2,v/v). Ten µl of the extract was chromatographed using a Shimadzu HPLC system (steel column, 150 x 4.6 mm, Zorbaks-ODS; column temperature 35 C; 236 nm; mobile phase: water-methanol-acetonitrile (5:3:2,v/v), flow rate, 1 ml/min) and zearalenone was identified and quantified based on the authentic sample (Fig. 2).

All the test samples (as indicated in Table 3) were dissolved in 0.1 ml of dimethyl sulphoxide (DMSO) and assayed for mutagenicity (Maron and Ames, 1983). Histidine deficient *Salmonella typhimurium,* tester strains TA98, TA1535, TA1537 and TA1538 were used. A mixture containing 0.5 ml of 0.02 M phosphate buffer (pH 7.4) or S9 (post-mitochondrial supernatant) mix, 0.1 ml of 16 h bacterial culture and 0.1 ml of DMSO/test substance in DMSO was incubated at 37 C for 20 min. Following incubation, 2 ml of molten agar (40-45 C) containing traces of histidine and biotin was added to it and poured over minimal glucose agar plates. The plates were incubated at 37 C for 72 h and revertant his^+ colonies were scored (Balachandran et al. 1990).

Groups of ten Swiss mice, 8-10 weeks old (25-30 g) were used for the experiments to study the chromosomal aberrations, micronuclei production and sperm head abnormalities. The test substance (dosage, mentioned in Tables 4-6) was administered orally along with the powdered chow diet, daily for 35 days. Water was provided *ad libitum.* Animals fed 20-methylcholanthrene served as positive control, while animals on normal diet served as the negative controls. Colchicine, 0.1 mg in 0.1 ml of water was administered intraperitoneally to each animal, 90 min before sacrifice, to arrest the metaphase. The femoral bones were removed after sacrifice and cleared off of all adhering muscle tissues. The bone marrow was aspirated into 7-8 ml of 0.9% saline and the contents were centrifuged for 5 min at 500 x g. The supernatant was removed and to the pellet, 6 ml of prewarmed (at 37 C) 0.075 M KCl was added. After incubating at 37 C for 25 min, the contents were again centrifuged at 500 x g for 5 min. The supernatant was discarded and the pellet was fixed in fresh methanol-acetic acid (3:1,v/v) fixative. The plates were observed for different types of aberrations and the per cent aberrant metaphases was calculated (MacGregor and Varley 1983).

Micronuclei produced in the bone marrow cells were evaluated following the method of Adler (1984). Micronuclei, dark stained and round in shape were scored in polychromatic erythrocytes (PCEs, immature erythrocytes, pink to purple in colour) and in normochromatic erythrocytes (NCEs-mature erythrocytes, yellowish in colour). Per cent micronuclei in these erythrocytes was calculated. Since several mutagens and carcinogens are responsible for the development of abnormal sperms, the abnormalities in the sperms of treated animals were recorded. Immediately after sacrifice, *cauda epydidymus* was removed from the animal and placed in 0.9% saline. It was minced finely and then left undisturbed for 30 min for the diffussion of spermatozoa (Wyrobek and Bruce 1978). The spermatozoa were spread on microscopic slides, air dried, fixed in absolute methanol and then stained with 1% eosin yellow. One thousand sperm from each animal were examined for abnormalities.

RESULTS AND DISCUSSION

Chrysene was detected in almost all the pyrolysed samples (Table 1). Salted, sundried and oil fried ribbon fish contained chrysene as high as 18.57 µg/g, while the decoction prepared from cumin seeds, mint leaves and cardamom had 12.61 µg/g. Dibenzanthraccene (DBA) was also high in the decoction. Salted and fried *Capsicum*

annum had 27.48 µg/g of DBA, while whitebait fish contained 12.34 µg/g. Benzo(a)pyrene (BP) was detected in *Cyamopsis tetragonaloba* and in all the sundried fishes. Oil used for frying (heated oil) did not reveal any PAH. Raw food components, such as pepper, cumin seeds, mustard and ginger revealed chrysene, while aniseeds contained DBA, in addition to chrysene (Table 1). Of the nineteen samples screened, only five revealed the presence of zearalenone and the results are presented in Table 2.

The mutagenicity of the food items and food components is depicted in Table 3. All the items are widely used and most of them were mutagenic. Palm drink and 'toddy', which are commonly used local beverages were also mutagenic. Salted, sundried and oil fried vegetables and fishes, as well as calamus oil induced high frequency of chromosomal aberrations (aberrant metaphases), almost comparable with the positive control (Table 4). The aberrations were moderate in fried mustard, pyrolysed cumin seeds and aniseeds and in *Cissus quadrangularis* treatments. A similar trend was observed in micronuclei assay (Table 5). Salted, sundried and oil fried vegetables and fishes and calamus oil developed more of micronuclei. Per cent micronuclei was low in palm drink and toddy treatments. Results concerning the production of sperm head abnormalities by the test substances are presented in Table 6. The pyrolysed vegetables and fishes were most effective, while other samples were either moderately effective or non-effective.

All the test samples are widely consumed in South India. DMSO extracts of the pyrolysed vegetables and fishes displayed appreciable mutagenic activity and this can be attributed to the presence of PAHs. We have identified and quantified BP, chrysene and DBA in the pyrolysed food items. Presumably, these are formed during deep frying and cooking. A series of heterocyclic amines and aromatic hydrocarbons from the pyrolysates and ordinary cooked foods have been quantified by Kasai et al. (1990). Heterocyclic amines are also isolated from broiled sardines which are carcinogenic in various organs (Kosuge et al. 1978). Further, most of them are carcinogenic in two or more organs (Sugimura 1986). The perturbing point is the detection of PAHs in raw and uncooked food components of South India, suggesting that most of the items are derived from the polluted areas. Interestingly, PAHs are also reported in vegetables (Corning and Lansdown 1983). Most of the food items, we have screened are mutagenic as revealed through Ames assay. The most probable causative agents, PAHs, are detected in this study. Evidently, PAHs present in alarming quantities in the commonly and widely consumed food items may play a major role in the high incidence of gastric cancers of South India.

Calamus oil, which is widely used in perfumes and pharmaceuticals, contains β-asarone in appreciable amounts and this can be attributed to its mutagenic and genotoxic effects. β-Asarone induces chromosomal aberrations in human lymphocytes and slight increase in the rate of sister chromatid exchange (Abel 1987). Similarly, allylisothiocyanate, a major ingredient of mustard causes chromosomal aberrations in hamster cells and is carcinogenic in rats. Ascorbic acid, mutagenic at higher doses (Stich et al. 1980), is present in the extracts of *Cissus quadrangularis*. Although genotoxicity of ascorbic acid is not known, its presence in *Cissus* may attribute to the genotoxic effects. Mutagens

Table 1

Polynuclear Aromatic Hydrocarbons (PAHs) in foods and medicinal preparations ("Decoctions")

Food item	PAH	Concentration (µg/g)
Dry-Heated (Pyrolysed) spices:		
Pepper	Chrysene	0.106
Cumin seeds	Chrysene	0.020
	DBA	0.509
Aniseeds	Chrysene	0.102
Cardamom	None	-
Mint leaves	None	-
Pyrolysed portion of :		
Chappatti (wheat flour item)	BP	0.676
	Chrysene	0.938
	DBA	3.695
Bread toast	BP	0.226
	Chrysene	0.282
Cutlet	BP	1.418
Calamus	BP	0.008
	DBA	0.129
Oil-fried, salted & sun dried vegetables:		
Chilles *(Capsicum annum)*	Chrysene	1.995
	DBA	27.480
Cluster beans *(Cyamopsis tetragonaloba)*	Chrysene	0.965
	DBA	9.540
Sundakkai *(Solanum torvum)*	BP	0.060
Oil-fried, salted & sun dried fishes:		
Whitebait fish *(Stolephorus bataviensis)*	Chrysene	4.280
	DBA	12.340
	BP	5.730
Ribbon fish *(Trichiures leptures)*	Chrysene	18.570
	BP	60.170
Seer fish *(Scomberomorus ccommersonnii)*	Chrysene	3.570
	BP	10.290
Sun-dried fishes:		
Ribbon fish	BP	0.105
Seer fish	DBA	0.409
Whitebait fish	None	None
Decoction made from pyrolysed:		
Cumin Seeds + Aniseeds	Chrysene	0.520
Cumin Seeds + Aniseeds + dry ginger	Chrysene	2.011
Cumin Seeds + mint leaves + cardamom	Chrysene	12.610
	DBA	31.150
	BP	6.400
Pepper	Chrysene	0.057
Cumin seeds	Chrysene	0.011
Aniseeds	Chrysene	0.020
	DBA	0.302
Mustard	Chrysene	0.035
Ginger	Chrysene	0.072
Sundakkai *(Solanum torvum)*	DBA	0.621
Chillies	None	None
Cluster beans	None	None
Heated oil (medium used for frying)	None	-
Fried Mustard	Chrysene	0.070
Pyrolysed portion of "Uppuma"	Chrysene	2.650
Cardamom	None	None
Mint leaves	None	None

BP : 3, 4 Benzo (a) pyrene ; DBA : 1,2,5,6 Dibenzanthracene

Table 2. Zearalenone in South Indian Food components

Food Component	Zearalenone
Maize	1.4536 mg/kg
Wheat	4.7440 mg/kg
Ragi	2.0560 mg/kg
Toddy	0.7652 mg/l
Milk	0.0248 mg/l

In buttermilk, cashew nut, coconut, ground nut, areca nut, semolina, rice, tomato, coriander, tamarind, curry leaves, aniseeds, ginger and mint leaves, zearalenone was not detected.

Table 3 Mutagenicity of Foods and Food components

Food Item	*Salmonella typhimurium*			
	TA 98	TA 1535	TA 1537	TA 1538
Oil fried, Salted & sun-dried vegetables :				
Sundakkai (*Solanum torvum*)	++	+++	+++	+++
Chillies (*Capsicum annum*)	+++	+++	+++	+++
Cluster beans *(Cyamopsis tetragonaloba)*	++	++	+++	+
Oil fried, salted & sun-dried fishes :				
Whitebait fish	+++	-	+++	++
Seer fish	+++	+	+++	+++
Ribbon fish	+++	+++	+++	+++
Decoctions prepared from pyrolysed :				
Cumin seeds + Aniseeds + Ginger	+	+	+	+++
Ginger + Pepper	-	-	+	-
Cumin seeds + Mint leaves + Cardamon	-	-	++	-
Charred portions of :				
Bread toast	++	-	++	++
Cutlet	++	-	++	+++
Calamus	+++	-	++	+++
Essences :				
Almond	+	-	+++	+
Kewra	+++	-	+++	+++
Rose	++	-	++	+
Vanilla	-	-	+++	+
Pickles :				
Sweet mango	++	-	+	+
Lemon	++	-	+	++
Mango	++	-	+	+
Raw dried fishes :				
Whitebait fish	+	-	+	+
Seer fish	+	-	+	+
Ribbon fish	+	-	+	+
Tamarind	+	-	+	++
Fried mustard	+	-	+++	+
Asafoetida	+	-	+++	+
Turmeric	-	++	+	+
Onion	+	++	+++	++
'Green' camphor	-	++	+	++
Kesari powder (Metanil yellow)	+++	+++	+++	+++
Palm oil	-	-	++	-
Calamus oil	-	-	+++	-
Perandai *(Cissus quadrangularis)*	-	-	+++	-
Palm drrink	-	-	+++	-
Toddy	-	-	+++	-
Arrack	-	-	+	-
Nutrine chocolate	-	-	+	=
Cinnamom zeylanicum	+++	-	++	++
Peppermint (Sweet)	+++	-	+++	+
BENZO (A) PYRENE	+++	-	++	++
CHRYSENE	+++	-	+++	++
DIBENZANTHRACENE	+++	-	++	+

+++ Mutagen : Revertant colonies, more than 100% of the control
++ Weak Mutagen : Revertant colonies, more than 75% of the control
+ Non-mutagen ; - Not yet done

Table 4

Frequency of chromosomal aberrations in bone marrow of mice treated with different foods

Food tested	Dose (g/day/ animal)	No. of meta-phases	Chromatid gaps	Chromatid breaks	Chromatid exchan-ges	Rings	Hypo- & hyper-ploidy	Frag-ments	Per cent aberrant metaphases
20-Methylcholanthrene (Postive Contrrol)	0.005	50	8	6	2	2	3	3	48 ± 1.16
Normal diet (Negative Control)		50	1	-	-	-	-	-	2 ± 0.58
Salted sundried & oil fried items:									
Solanum torvum	0.1	50	6	5	2	-	4	3	40 ± 0.57
Cyamopsis tetragonaloba	0.1	50	9	4	2	1	4	1	42 ± 2.31
Stolephorus bataviensis	0.1	50	5	9	4	1	1	2	44 ± 4.05
Scomberomorus commerssonnii	0.1	50	7	6	3	-	3	-	38 ± 1.16
Trichiures lepturus	0.1	50	7	8	3	-	-	2	40 ± 4.62
Frried Mustard	0.1	50	5	3	1	1	3	1	30 ± 3.50
Calamus Oil	0.005 ml	50	11	4	5	-	1	1	42 ± 3.47
Toddy	0.1 ml	50	4	2	-	-	3	1	20 ± 4.05
Palm drink	0.1 ml	50	3	2	-	-	1	2	16 ± 1.73
Cumin seeds+Aniseeds (Pyrolysed)	0.1	50	3	2	4	1	-	3	26 ± 1.15
Cissus quadrangularis	0.1	50	4	3	2	-	3	2	28 ± 0.57

Table 5

Incidence of micronucleated bone marrow cells in mice after treatment with various foods

Food tested	Dose (g/day/ animal)	Percentage of polychromatic erythrocytes micronucleated	Percentage of normochromatic erythrocytes micronucleated
20-Methylcholanthrene (Positive control)	0.005	1.96 ± 0.09	0.67 ± 0.09
Normal diet (Negative Control)		0.20 ± 0.06	0.13 ± 0.03
Salted, sundried & oil fried items :			
Solanum torvum	0.1	1.63 ± 0.03	0.33 ± 0.02
Cyamopsis tetragonaloba	0.1	1.53 ± 0.09	0.50 ± 0.06
Stolephorus bataviensis	0.1	1.50 ± 0.25	0.53 ± 0.14
Scomberomorus commerssonnii	0.1	1.76 ± 0.14	0.73 ± 0.12
Trichiures lepturus	0.1	1.33 ± 0.02	0.46 ± 0.14
Fried mustard	0.1	1.20 ± 0.06	0.37 ± 0.02
Calamus oil	0.005 ml	1.60 ± 0.17	0.80 ± 0.12
Toddy	0.1 ml	0.67 ± 0.12	0.23 ± 0.09
Palm drink	0.1 ml	0.80 ± 0.06	0.27 ± 0.03
Cumin seeds+Aniseeds (Pyrolysed)	0.1	1.16 ± 0.11	0.40 ± 0.05
Cissus quadrangularis	0.1	1.06 ± 0.20	0.46 ± 0.14

Table 6

Sperm head abnormalities in mice induced by various foods and food components

Food tested	Dose (g/day/ animal)	Total No. of sperms studied	Total No. of abnormal sperms	Percentage of abnormal sperms
20-Methylcholanthrene (Positive control)	0.005	1000	177	17.7 ± 0.46
Normal diet (Negative control)		1000	18	1.8 ± 0.20
Salted, sundried & oil fried :				
Solanum torvum	0.1	1000	135	13.5 ± 0.49
Cyamopsis tetragonaloba	0.1	1000	125	12.5 ± 0.56
Stolephorus bataviensis	0.1	1000	141	14.1 ± 1.39
Scomberomorus commerssonnii	0.1	1000	152	15.2 ± 0.81
Trichiures lepturus	0.1	1000	128	12.8 ± 0.75
Fried mustard	0.1	1000	107	10.7 ± 0.34
Calamus oil	0.005 ml	1000	139	13.9 ± 1.44
Toddy	0.1 ml	1000	69	6.9 ± 0.17
Palm drink	0.1 ml	1000	53	5.3 ± 0.34
Cumin seeds+aniseeds (Pyrolysed)	0.1	1000	103	10.3 ± 0.28
Cissus quadrangularis	0.1	1000	112	11.2 ± 0.57

present in alcoholic beverages and other drinks are poorly absorbed in mammals (Bird et al. 1982). The minimal effect of toddy and palm drink on the genetic material, is probably due to the non-availability of the critical concentration of the genetically reactive metabolites at the target molecules (DNA and protein) (Tates and Natarajan 1976). The micronucleus test is comparably more sensitive and reliable than metaphase scoring, in the screening of chemical agents for mutagenicity (Schmid 1978). The relevance of sperm head abnormality in identifying mutagens is well accepted (Wyrobek et al. 1983). It is generally argued that sperm head abnormalities primarily arise from point mutations, rather from gross chromosomal change (Wyrobek and Bruce 1978).

There is often a correlation between *in vitro* mutagenicity and *in vivo* carcinogenicity (Lijinsky 1989). In the present study, most of the food items are mutagenic. Admittedly, these food substances are potentially carcinogenic. Further, it is clear from the results, that the concentration of zearalenone in the commonly consumed food stuffs is quite appreciable and daily uptake of these items, may definitely, cause adverse effects. Generally, zearalenone induces enlarged mammary glands (Mirocha et al. 1977), reduced fertility in cattle, fertility disturbances and foetal skeleton anomalies (Ruddick et al. 1976). Evidently, the South Indian food items are contaminated and cause mutagenic and genotoxic effects. However, it is yet to be proved, whether a repetitive dietary intake of small doses of both known and unknown environmental mutagens and carcinogens would contribute to the gastrointestinal cancer in and around Madras.

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