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Research Article
Antimutagenic Activity of Turmeric Extract Beverages

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Abstract

Objective: This study was conducted to investigate the potential of turmeric extract beverages to reduce the risk of mutagenic contaminants usually found in grilled and smoked food. **Methodology:** Turmeric extract beverages containing varying concentrations of curcuminoid were evaluated by a panel of judges. From overall "liking" scores, two concentrations were selected for the next study. The effects of both pasteurization and sterilization as well as storage times on the curcuminoid content of the two selected concentrations were measured. The antimutagenic potential of these two concentrations against two groups of mutagens, heterocyclic amines (Trp-P-1, Trp-P-2 and PhIP) and polycyclic aromatic hydrocarbons (BaP), was tested using the Ames test with *Salmonella typhimurium* strains TA 98. **Results:** Turmeric extract beverages with 400 and 800 μg curcuminoid/100 mL were determined to be acceptable for further study. Pasteurization and sterilization processes did not affect the pH or color of the turmeric extract beverages. The curcuminoid content in the sterilized beverages decreased more rapidly than in the pasteurized beverages during storage. After storage for 8 weeks, the mutagenic inhibition percentages decreased from the original values (before heating) but they were still ranked as active antimutagens. **Conclusion:** Turmeric extract beverages demonstrated antimutagenic activity against Trp-P-1, Trp-P-2, PhIP and BaP and has the potential for use as an alternative product for cancer prevention.

Key words: Turmeric beverage, antimutagenic activity, curcuminoid, heterocyclic amines, polycyclic aromatic hydrocarbons

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food is a source of anti-free radical substances, antimutagenic and anticancer compounds, however, it is also contaminated with mutagens and carcinogens. Mutagen contamination may result from the cooking process. Meat grilled or roasted at high temperature produces carcinogens, including nitrosamine and Polycyclic Aromatic Hydrocarbons (PAHs) that are produced by the burning of fat and Heterocyclic Aromatic Amines (HAAs) that are produced from the breakdown of proteins and amino acids[1]. When meat is grilled on a charcoal fire, fat drips down onto the coals, causing oil breakdown and a chemical reaction that results in the formation of PAH, which then rises in the smoke and is deposited on the surface of the meat. The PAHs, such as benzo[a]pyrene (BaP) and benzo[a]anthracene, have been shown to be carcinogenic in animal experiments. The Phe-P-1 and Orn-P-1 substances are formed from the burning of phenylalanine and ornithine. The IQ and MeIQ, resulting from the combustion of creatinine and sugar in grilled sardines, have much higher mutagenicity than aflatoxin B1[2] and have caused cancer in the liver, lungs, stomach, upper intestine and blood vessels in animal experiments[3]. Smoked foods such as sausage, ham and bacon, contain BaP. Roasted pork and grilled chicken are often prepared by marinating with monosodium glutamate (MSG) and seasoning sauces. When these forms of meat are grilled or baked at high temperatures, glutamate in the MSG can be converted to Glu-P-1 and Glu-P-2, which are recognized carcinogens in the heterocyclic amines group and have been shown to cause gastrointestinal, liver and brain cancer[1,3]. Thus, scientists have long been interested in food as a cancer prevention tool. Substances in plant have the ability to inhibit mutation, which is the primary cause of cancer, by increasing the activity of enzymes that detoxify carcinogens before they breakdown and become toxic or by inhibiting cancer cells in the body after exposure to a carcinogen. Turmeric (*Curcuma longa* L.) is widely utilized in India, China and Southeast Asian countries because it has shown many medicinal benefits including anti-inflammatory, antioxidant, anticancer and anti-HIV properties. The chemical components of turmeric can be divided into two groups: volatile compounds, which give turmeric its smell and non-volatile compounds, which provide color. Curcuminoids are a large group of non-volatile phenolic compounds containing three chemicals: Curcumin 60-70%, demethoxycurcumin and bisdemethoxycurcumin. These compounds are all easily degraded by light and hydrolytic reactions[4]. Turmeric does not dissolve in water but it is soluble in alcohol and acetic acid. Surojanametakul *et al.*[4] recently prepared curcuminoid powder from turmeric as a ready-to-use extract and tested it for mutagenicity and toxicity. Their results showed no mutagenicity and no toxicity to the genes of an organism. This study was aimed to investigate the potential of turmeric extract beverages using ready-to-use turmeric extract powder to reduce the risk of mutagenic contaminants from grilled and smoked food.

MATERIALS AND METHODS

Preparation of turmeric extract powder: Turmeric rhizomes were purchased from a market in Ratchaburi Province. After cleaning with water and steaming at 100°C for 7 min, the rhizomes were processed into slices 0.1-0.2 cm thick, placed on grill trays and dried at 60°C for 12 h in a hot air oven. After fine grinding using a blender, the resultant powder was sifted through a 60-mesh sieve and stored in an airtight container until required for use. Curcuminoid was extracted by dissolving turmeric powder in a 1:1 ethanol/water mixture at 50°C for 2 h. The procedure of Surojanametakul *et al.*[4] was then followed. The extract yield was 35-39% and the curcuminoid comprised 14.63±0.38% of the turmeric extract. The yellow turmeric extract powder contained 41.21 mg curcuminoid/100 g dry basis; thus, the beverage made from 1 g of powder of 100 mL of water contained 412.1 µg curcuminoid.

Preparation of turmeric extract beverage: Because turmeric extract in water has an unpalatable taste, fruit flavoring, citric acid and sugar were added. Four concentrations of turmeric beverage were prepared with 0.5, 1, 2 and 3% turmeric which contained 200, 400, 800 and 1200 µg curcuminoid/100 mL, respectively (Table 1). Pasteurization (90°C for 7 min) and sterilization (121°C for 10 min) were used for thermal processing. The bottled turmeric extract beverages were kept at 25±2°C in the laboratory.

Chemicals and media: Two groups of standard mutagens were used: a) heterocyclic amine group containing 3-amino-1,4-dimethyl-5H-pyrido[4,3-6] indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-b] indole (Trp-P-2) (Wako

Table 1: Four concentrations of the turmeric extract beverages

	Beverages			
Ingredients	R_1	R_2	R_3	R_4
Turmeric extract powder	0.50	1.00	2.00	3.00
Sucrose	8.00	8.00	8.00	8.00
Fructose	6.00	6.00	6.00	6.00
Citric acid	0.50	0.50	0.50	0.50
Pineapple flavor	0.20	0.20	0.20	0.20
Water	84.80	84.30	83.30	82.30

Pure Chemical, Japan) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and b) polycyclic aromatic hydrocarbon group containing benzo[a]pyrene (BaP) (Sigma, USA). Standard curcumin with a purity in excess of 98% and analytical grade dimethyl sulfoxide (DMSO) were purchased from Sigma (USA) and S9 mix enzyme was purchased from Kikkoman (Japan). All other chemicals at analytical grade were purchased from BDH Chemicals (UK). Analytical grade media used were Bacto agar (Scharlau Chemicals, Spain), Nutrient Broth No.2 (Oxoid, UK) and top agar (agar containing 10% 0.5 M L-Histidine–HCl-0.5 mM biotin mixture) (Sigma-Aldrich, UK).

Microorganisms: A *Salmonella typhimurium* specific strain without a gene necessary for the production of histidine, TA 98 (the strain was manipulated as suggested by Maron and Ames[5], provided as a courtesy by Dr. Wannee Kusamran (National Cancer Institute, Ministry of Public Health, Thailand), was used in the antimutagenic studies describes below. Stock bacteria were cultured on Oxoid Nutrient Broth No. 2 at 37°C in the presence of oxygen for 16 h prior to each experiment.

Sensory evaluation of turmeric extract beverage: Four turmeric extract beverage samples heated in a pasteurizer were evaluated for appearance, odor, flavor and overall "liking" by 30 panelists using a 9-point hedonic scale with score 1 = Dislike extremely, 2 = Dislike very much, 3 = Dislike moderately, 4 = Dislike slightly, 5 = Neither like nor dislike, 6 = Like slightly, 7 = Like moderately, 8 = Like very much and 9 = Like extremely. The two samples that received the highest overall "liking" scores were selected for further studies.

Sensory evaluation and physical properties of the selected turmeric extract beverages: Two turmeric beverage recipes, each prepared by 2 different thermal processes (for a total of 4 samples), were evaluated for sensorial properties using a 9-point hedonic scale. The pH values were measured using a pH meter (PB-20, Germany). The color (L*, a* and b* values) was determined using a Spectraflash SF600 Plus (Datacolor International, USA).

Curcuminoid determination of the selected turmeric extract beverages during storage: A sample of the selected turmeric extract beverage (0.05 g) and 130 mL of 95% ethanol were combined in a 250 mL round bottomed flask and run in a Soxhlet extraction for 2.5 h to extract curcumonoid. After filtrating through Whatman No. 4 paper, the filtrate volume was adjusted to 100 mL with 95% ethanol. Then, 20 mL of this solution was transferred by pipet to a 250 mL volumetric flask and 95% ethanol was added. The curcuminoid concentration was measured by the absorbance at 425 nm and 95% ethanol was used as the blank.

Antimutagenic activity determination of the selected turmeric extract beverages: The mutagenic activity determination of standard mutagen followed a preincubation technique[6] modified from the Ames assay. *Salmonella typhimurium* TA98 was exposed to a standard mutagen; Trp-P-1, Trp-P-2, PhIP, or BaP at concentrations of 42, 50, 314 and 2,100 ng per test tube, respectively. The preincubation mixture (0.1 mL) was added with 0.7 mL of 0.1 M phosphate buffer (pH 7.0), 0.05 mL of turmeric extract (various concentration), 0.1 mL of the enzyme S9 mix and adjusted to the final volume of 1.0 mL with distilled water. The mixture was incubated at 37°C in a shaking water bath (Memmert SV1422, Germany) for 20 min. After mixing with top agar containing histidine and biotin and pouring onto a glucose agar plate prepared at least three days in advance, the samples were incubated at 37°C for 48 h. The average colony count of each sample was measured in two plates and the experiment was performed in two replicates. The DMSO was used as a solvent control to measure the mutagen-independent mutation rate of bacteria. The evaluation criterion for antimutagenic activity against standard mutagens was whether the extract samples showed any difference in mutagenicity (by Ames test) reduction when compared to a sample with no extract, indicating that the extract could inhibit mutations caused by standard mutagens. The antimutagenicity of each sample was obtained by the following Eq. 1[7]:

$$\text{Inhibition (\%)} = \frac{C_0 - S}{C_0 - C_{100}} \quad (1)$$

where, C_0 is the number of bacteria colonies mutated by standard mutagen solutions without extract samples (positive control), C_{100} is the number of bacterial colonies mutated in a mutagen-independent way (negative control) and S is the number of bacterial colonies mutated by standard mutagen solutions with extract samples.

The ability of the turmeric extract samples to inhibit mutation was determined using the criteria of Kruawan and Kangsadalampai[7] where more than 60% inhibition means strongly active, 60-41% inhibition means active, 40-21% inhibition means weakly active and 20-0% inhibition means not active.

Statistical analysis: Data were analyzed using one-way analysis of variance followed by Duncan's New Multiple Range Test (DNMRT) for comparisons among means with a significance level of 5%. Statistical analysis was performed using SPSS 16 software (SPSS Inc., USA).

Table 2: Sensory evaluation of the turmeric extract beverages at different concentrations

Curcuminoid (µg/100 mL)	Appearance	Odor	Flavor	Overall liking
200	6.88±1.41[a]	6.45±1.29[a]	6.53±1.85[a]	6.51±1.66[a]
400	6.82±1.37[a]	6.30±1.56[a]	6.11±1.44[a]	6.43±1.29[a]
800	6.71±1.52[a]	6.12±1.38[a]	5.97 ±1.16[a]	6.08±1.42[a]
1200	6.34±1.67[a]	5.29±1.84[b]	5.16±1.63[b]	5.27±1.75[b]

Means in the same column with different superscript letters are significantly different (p<0.05) by DMRT

Table 3: Sensory evaluation of the selected turmeric extract beverages by different thermal processes

Turmeric beverages	Thermal process (Temp/time)	Appearance	Odor	Flavor	Overall liking
R2	90°C/7 min	6.81±1.30[a]	6.20±2.01[a]	6.23±1.56[a]	6.38±1.67[a]
	121°C/10 min	6.65±1.41[a]	6.26±1.63[a]	6.14±1.70[a]	6.41±1.33[a]
R3	90°C/7 min	6.85±1.37[a]	6.08±1.56[a]	5.87±1.44 [a]	6.13±1.39[a]
	121°C/10 min	6.71±1.52[a]	5.89±1.38[a]	5.80±1.16[a]	6.09±1.42[a]

Means in the same column with different superscript letters are significantly different (p<0.05) by DMRT

Table 4: Physical properties of bottled turmeric extract beverages

			Color		
Turmeric beverage	Thermal process (Temp/time)	pH	L*	a*	b*
R2	90°C/7 min	3.67	93.52[a]	-7.03[a]	26.85[b]
	121°C/10 min	3.68	95.44[a]	-7.83[a]	27.12[b]
R3	90°C/7 min	3.73	86.83[b]	-9.61[b]	46.07[a]
	121°C/10 min	3.73	89.06[b]	-9.85[b]	47.66[a]

Following the CIE LAB system, L*: Indicates lightness from 0 (black) to 100 (white), a*: Indicates redness (+a*) to greenness (-a*) and b* indicates yellowness (+b*) to blueness (-b*). Means in the same column with different superscript letters are significantly different (p<0.05) by DMRT

RESULTS AND DISCUSSION

Sensory evaluation of the turmeric extract beverages: To select the most suitable concentrations of curcuminoid in the beverage samples, both the antimutagenicity and sensory test results were considered. The results showed that all turmeric extract beverages R1, R2, R3 and R4 were acceptable, with overall "liking" scores ranging from 6.08-6.51, corresponding to 'like slightly' to 'like moderately' (Table 2). Appearance, odor, flavor and overall "liking" scores of beverages R1, R2 and R3 were not significantly different ($p \geq 0.05$). Beverage R4, which contained 1200 mg curcuminoid/100 mL, showed significantly lower scores (5.16-5.29) for odor, flavor and overall "liking" ($p<0.05$). The R1 contained the least amount of turmeric extract powder and had a relatively low percentage of mutation inhibition: however, its sensory scores were not significantly different from beverages R2 and R3. Siriwan *et al.*[8] prepared turmeric extract solutions contained 200, 400, 800 and 1200 µg curcuminoid/100 mL by dissolving 0.5, 1.0, 2.0 and 3.0 g of turmeric extract powder, respectively, in 100 mL of hot water (90°C). They found that the solution containing 200 µg curcuminoid/100 mL showed weakly active inhibition (28.41-32.98%) against Trp-P-1, Trp-P-2, PhIP and BaP. Thus, beverages containing 400 and 800 µg curcuminoid/100 mL (R2 and R3) were selected for further experiments, since R1 had weakly active mutation inhibition and R4 had the lowest sensory evaluation scores ($p<0.05$).

Sensory evaluation and physical properties of the selected turmeric extract beverages: Turmeric extract beverage R2 (400 µg curcuminoid/100 mL) and R3 (800 µg curcuminoid/100 mL) were prepared and heated using pasteurization and sterilization. The sensory characteristics of the 2 different turmeric beverages were not significantly different in terms of appearance (6.71-6.85), odor (5.89-6.26), flavor (5.80-6.23) and overall "liking" score (6.09-6.41) ($p \geq 0.05$) (Table 3). The turmeric extract beverage samples heated using pasteurization versus sterilization showed no pH difference (3.67-3.73) ($p \geq 0.05$) (Table 4). The L* values were not different between the pasteurized samples versus the sterilized samples ($p \geq 0.05$). The L* values of R2 (93.52-95.44) were lighter than R3 (86.83-89.06) whereas a* and b* value of R2 were less than R3 ($p<0.05$). This was expected since R3 contained more curcuminoid, which caused greater red and yellow coloration. Neither thermal process affected the pH or color of R2 or R3. Sterilization resulted in lighter, greener and yellower beverages than pasteurization. The greater color intensity was likely due to the Maillard reaction among the aldehyde or ketone group of reducing sugars and amino groups of amino acids, peptides or proteins

giving a final product with melanoidins[9]. Chuaynual and Rotsatchakul[10] found a similar result during sterilization of gac (*Momordica cochinchinensis*) fruit beverage and stated that the sterilization temperature generated a Maillard reaction during processing.

Curcuminoid concentration of the selected turmeric extract beverages: Pasteurization and sterilization appeared to affect the curcuminoid levels of turmeric extract beverages R2 (400 μg curcuminoid/100 mL) and R3 (800 μg curcuminoid/100 mL). For freshly processed turmeric extract beverages (at 0 week), the curcuminoid concentration of sterilized beverages R2 and R3 was significantly lower than that in the pasteurized beverages ($p<0.05$) (Table 5). Suresh *et al.*[11] reported that curcuminoids in spices containing turmeric were reduced by 1-30% after heating under high pressure. A comparison of curcuminoid concentration in turmeric extract beverage R2 stored for eight weeks versus freshly processed beverage showed a 34.4% decrease in the pasteurized sample and a 34.8% decrease in the sterilized sample. In beverage R3, curcuminoid concentration decreased significantly by 20.6 and 24.1% in the sterilized sample and the pasteurized sample, respectively ($p<0.05$). The reduction in beverage curcumonoid concentration was possibly caused by light, as the samples were stored in clear glass bottles. These findings were consistent with Kumavat *et al.*[12], who reported that curcumin was stable in acidic solution because of conjugated diene structure, while curcumin content decreased in neutral-basic condition. However, curcumin decomposed by more than 40% when a solution at pH of 1.2 was exposed to light.

Table 5: Curcuminoid concentration in turmeric extract beverages R2 and R3 during storage at 25°C for eight weeks

Thermal process (Temp/time)	Duration (week)	Curcuminoid (μg/100 mL) Beverage R2	Beverage R3
Pasteurization	0	413.17^{a}	822.31^{a}
(90°C/7 min)	1	412.53^{a}	809.81^{cd}
	2	406.49^{ab}	801.55^{e}
	3	380.23^{c}	805.46^{de}
	4	378.23^{cd}	786.23^{f}
	6	327.37^{g}	751.70^{h}
	8	271.25^{j}	652.50^{l}
Sterilization	0	379.39^{c}	805.12^{de}
(121°C 10 min)	1	380.84^{c}	788.45^{f}
	2	374.31^{cde}	781.08^{f}
	3	369.97^{de}	771.26^{g}
	4	339.79^{f}	743.03^{i}
	6	318.38^{h}	738.96^{i}
	8	247.26^{k}	611.04^{m}

Means in the same column with different letters are significantly different ($p<0.05$) by DMRT

Antimutagenicity of the selected turmeric extract beverages: Before heating, beverages R2 and R3 showed 38.74-59.28% and 41.54-62.60% mutagenic inhibition (data not shown), respectively. This percentage range is ranked as "active" according to the criteria of Kruawan and Kangsadalampai[7]. At week eight of storage, the mutagenic inhibition percentages were 29.16-41.57% (weakly active) and 36.77-60.68% (active) for of beverages R2 and R3, respectively (Table 6), which is lower than their original values (before heating). All tested mutagens, namely, Trp-P-1, Trp-P-2, PhIP and BaP, were reported as pro-mutagens/pro-carcinogens requiring metabolic activation for DNA-adduct formation. Previous investigations specified cytochrome P4501A2 as the catalytic enzyme essential for bio-activation of these promutagens[13,14]. Curcumin has been reported to inhibit various cytochrome P450 enzymes, including cytochrome P4501A2[15-18]. Therefore, turmeric extract beverages appear to possess the potential to inhibit cooked food mutagens, as it may inhibit the metabolic activation process of these mutagens. A methoxy group, hydroxy group, or alkene portion on the structure of curcumin and its derivatives (Fig. 1) play a key role in mutation inhibition by donating electrons to electrophilic metabolites resulting from enzyme-promutagen reactions[19]. Thus, turmeric extract beverages appear to have

Table 6: Mutation inhibition of turmeric extract beverages R2 and R3 after eight weeks of storage

	Inhibition (%)			
	Beverage R 2		Beverage R 3	
Mutagens	Pasteurization (90°C/7 min)	Sterilization (121°C/10 min)	Pasteurization (90°C/7 min)	Sterilization (121°C/10 min)
Trp-P-1	36.45^{b}	32.08^{c}	47.72^{c}	46.68^{c}
Trp-P-2	41.57^{a}	39.33^{a}	60.68^{a}	60.12^{a}
PhIP	38.19^{a}	36.69^{ab}	52.35^{b}	50.23^{b}
BaP	31.24^{c}	29.16^{c}	39.57^{d}	36.77^{d}

Trp-P-1:3-amino-1,4-dimethyl-5H-pyrido[4,3-6]indole, Trp-P-2: 3-amino-1-methyl-5H-pyrido[4,3-b]indole, PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine BaP: benzo[a]pyrene, Means of beverage R2 and R3 with different letters are significantly different ($p<0.05$) by DMRT

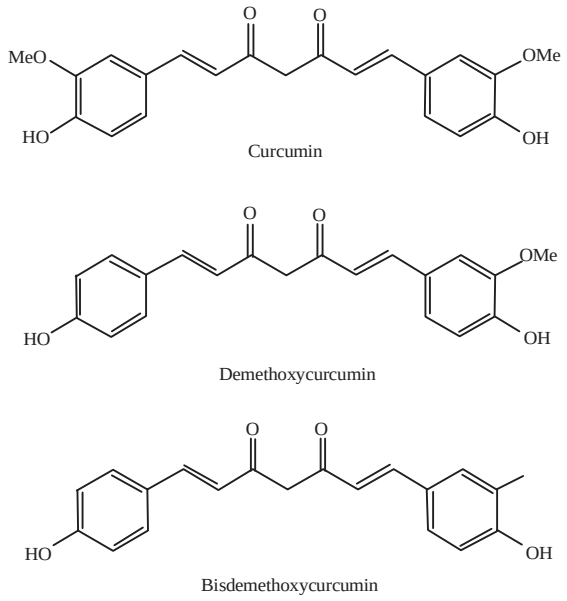

Fig. 1: Structure of curcumin and its naturally occurring derivatives: demethoxycurcumin (curcumin II) and bisdemethoxycurcumin (curcumin III)

the ability to protect DNA from the intermediate metabolites of mutagens Trp-P-1, Trp-P-2, PhIP and BaP.

CONCLUSION

Turmeric extract beverage prepared from ready-to-use turmeric extract powder showed mutagenic inhibition potential for frameshift mutation caused by the following four mutagens: Trp-P-1, Trp-P-2, PhIP and BaP. The sterilization process resulted in a greater curcuminoid decrease than pasteurization and bottled turmeric extract beverage maintained antimutagenicity at weakly active to active levels after storage for 8 weeks. Thus, turmeric extract beverages could potentially be used as an alternative product for consumers that desire a beverage that may reduce risk of cancer.

SIGNIFICANCE STATEMENT

This study discovers the turmeric extract beverages contained curcuminoid with effective anti-mutagenic potential. These findings can be beneficial for consumers who would like to reduce risk of mutagens and carcinogens when they consume grilled and smoked food. The results from this study will help researchers to develop new processed food products contained turmeric extract powder that are easy to digest.

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