

Full Length Research Paper

Hypolipidemic and antioxidant potency of heat processed turmeric and red pepper in experimental Rats

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The hypocholesterolemic and antioxidant potency of both raw and pressure-cooked turmeric and red pepper were evaluated in experimental rats rendered hypercholesterolemic by feeding 0.5% cholesterol enriched diet and maintained for 8 weeks on 5% spice diet. Dietary turmeric and red pepper, either raw or heat processed significantly countered the extent of hypercholesterolemia. Serum total cholesterol was 31 and 32% lower as a result of feeding raw and heat processed turmeric. The same was lower by 16 and 23% in animal groups fed raw and heat processed red pepper. The reduction in blood cholesterol brought about by these two dietary spices was predominantly in the LDL-cholesterol fraction. Dietary red pepper, both raw and heat processed fully countered the increase in serum triglyceride content of hypercholesterolemic rats. Increase in hepatic cholesterol in hypercholesterolemic animals was moderately countered by dietary red pepper, either raw or heat processed. Both dietary turmeric as well as red pepper significantly countered the increase in hepatic triglyceride level in hypercholesterolemic rats. Total thiols in serum were slightly but significantly increased by raw turmeric and raw red pepper both in basal and in hypercholesterolemic rats, but not by heat processed spices. Serum α -tocopherol was significantly enhanced (81 - 113%) by both dietary turmeric and red pepper in hypercholesterolemic animals. Hepatic lipid peroxides were significantly lower (9 - 15%) as a result of dietary turmeric and red pepper in hypercholesterolemic situation. Thus, the results of this animal study suggested that although heat processing of turmeric and red pepper by pressure cooking resulted in a considerable loss of the active principles – curcumin and capsaicin, the hypolipidemic potency or the antioxidant potency of the parent spices were not significantly compromised.

Key words: Antioxidant effect, heat processing, hypocholesterolemic effect, red pepper, turmeric.

INTRODUCTION

Spices are a group of esoteric food adjuncts, which have been in use for thousands of years. By virtue of their pleasing colour, flavour or pungency, they can transform our food into attractive and appetizing meal. In addition to these organoleptic properties, few spices are also known to possess several medicinal properties (Nadkarni and Nadkarni, 1976) and are effectively used in the indigenous systems of medicine. In the past three decades, it has been experimentally documented that several common spices can also exert health beneficial physiological effects (Srinivasan, 2005). These physiological effects of

of spices in most instances have been attributed to the main spice active principles present in them. Among these physiological influences spices are documented to exhibit, their hypolipidemic and antioxidant properties have far-reaching health implication. The active principles of the spices - turmeric (*Curcuma longa*) and red pepper (*Capsicum annuum*) have been evidenced in several animal studies to exert hypolipidemic and antioxidant properties (Srinivasan, 2005).

It is also evidenced that heat processing of spices – turmeric, red pepper and black pepper results in a significant loss of their active principles (Srinivasan et al., 1992; Suresh et al., 2007). Hence, it is desirable to characterize the altered compounds formed from spice active principles during heat processing of parent spices, and to

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ascertain the extent to which these spices retain their health beneficial potency in spite of significant chemical alteration of active principles. Especially the hypolipidemic potency and the antioxidant potency exerted by turmeric and red pepper by virtue of their respective active compounds – curcumin and capsaicin need to be evaluated in heat-processed spices. In this investigation, elaborate animal studies were made to quantitate the relative potency of cooked turmeric and red pepper with regard to their hypolipidemic and antioxidant properties.

MATERIALS AND METHODS

Heat processing of spices

Spices – turmeric (*Curcuma longa*) and red pepper (*Capsicum annuum*) were locally purchased and powdered to pass through No.50 mesh sieve. These spice powders were suspended in distilled water (100 g / L) and subjected to pressure cooking for 10 min at 15 p.s.i. At the end of heat treatment, the samples were cooled to room temperature. Appropriate controls were also included wherein the samples did not undergo any treatment. These samples were lyophilized and stored at 4°C.

Quantitation of spice principles by TLC

Spice samples in the lyophilized food samples were extracted with ethyl acetate in a Soxhlet apparatus for 4 h. The extracts were concentrated in a flash evaporator to a known volume (2 ml) and stored in dark at -20°C until further analysis. The individual spice principles were quantitated after separation by appropriate TLC procedures as described below (Ravindranath et al., 1981; Srinivasan et al., 1981). Care was taken to minimize the exposure to light during the extraction procedure and TLC separation.

Curcumin

Aliquots of the ethyl acetate extracts (quadruplicate) of the lyophilized food material and reference curcumin (6 µg) were spotted on silica gel -G coated plates (20 X 20 cm). The plates were developed with the upper phase of the solvent system: benzene-ethanol-water-acetic acid (100: 27.5: 7.5: 0.5 v/v/v/v) in a chamber pre-equilibrated with the above solvent system for 2 h. The yellow curcumin bands were scraped off and quantitatively transferred to centrifuge tubes. Curcumin in the scrapings was extracted with 4 ml acetone, centrifuged at 2000 rpm for 5 min and 2 ml of the clear supernatant was used for the rubrocurcumin reaction. Two ml of acetonic extract was transferred to another test tube into which were successively added, 1 ml 7.5 mg% boric acid (in acetone) and 1 ml 5 mg% boric acid (in acetone). The contents of the tube were evaporated to dryness over a hot water bath. The residue was redissolved in 2 ml ethanol and absorption of the purple colour was measured at 550 nm.

Capsaicin

Aliquots of ethyl acetate extracts (quadruplicate) were spotted on silica gel -G coated plates (20 X 20 cm) along with reference capsaicin (40 µg). Plates were developed with petroleum ether (60 – 80°C) - acetone (65: 35 v/v) in a chamber pre-equilibrated with the same solvent for 90 min. The developed plates were air-dried and then sprayed uniformly with fresh Gibb's reagent (0.1% 2,6-

dichloroquinone-4-chlorimide in methanol). The plates, when dry, were exposed to ammonia vapours in a closed chamber for exactly 1 min. The blue-coloured zones of capsaicin thus visualized were scraped off and quantitatively transferred to centrifuge tubes containing 2 ml water. The tubes were vortexed for 10 min to extract the colour and centrifuged at 2000 rpm for 2 min. Absorption of the clear blue supernatants was read at 610 nm.

Animal treatment

Female Wistar rats (8 per group) weighing 110 - 120 g and housed in individual stainless steel cages were maintained on various experimental diets *ad libitum* for 8 weeks. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; NRC vitamin mixture, 1; Bernhardt-Tommarelli modified NRC salt mixture, 4, and refined peanut oil, 10. The hypercholesterolemic diet consisted of 0.5% cholesterol and 0.125% bile salts at the expense of an equivalent amount of corn starch in the basal diet wherein the peanut oil is also replaced with hydrogenated vegetable fat. The test material was incorporated into the basal diet/high cholesterol diet at 5 g / 100 g replacing an equivalent amount of corn starch. At the end of the experimental duration, overnight fasted animals were sacrificed under light ether anesthesia. Blood was collected by heart puncture and serum separated by centrifugation. Liver was quickly excised, weighed and stored frozen till lipid extraction.

Lipid profile

Total lipids were extracted according to Folch et al. (1957) and estimated gravimetrically. Cholesterol (Searcy and Bergquist, 1960), triglycerides (Fletcher, 1968) and phospholipids (Charles and Stewart, 1980) were determined in the lipid extracts of serum and liver by using standard procedures. Serum cholesterol and triglyceride associated with HDL fraction were determined after precipitation of apolipoprotein-B containing lipoproteins with heparin-manganese reagent according to the method of Warnick and Albers (1978). LDL-VLDL precipitate was extracted with chloroform: methanol (2:1 v/v) and used for cholesterol and triglyceride determination.

Lipid peroxides

Plasma lipid peroxides were estimated by the fluorimetric measurement of thiobarbituric acid complex by the method of Yagi (1984). The fluorimetric measurement was carried out at an excitation wavelength of 515 nm and emission wavelength of 553 nm and compared with the standards prepared by reacting 0.5 nmole 1,1,3,3-tetraethoxy-propane with TBA reagent. Lipid peroxide in liver tissue was determined by the method described by Ohkawa et al. (1979) involving photometric measurement of thiobarbituric acid complex extracted into butanol. Absorbance of the butanol extract was measured at 532 nm and compared with that of standard tetraethoxypropane, treated similarly.

Antioxidant molecules

Total thiols in serum/liver were measured spectrophotometrically by using Ellman's reagent according to the method described by Sedlock and Lindsay (1968). Glutathione in liver was estimated by using Ellman's reagent according to Beutler et al. (1963). Ascorbic acid in serum/liver was estimated spectrophotometrically by measuring the 2,4-dinitrophenyl-hydrazone derivative of dehydro-ascorbic acid according to Omaye et al. (1973). α -Tocopherol in serum was determined by HPLC method described by Zaspel and

Table 1. Influence of dietary turmeric and red pepper on serum lipid profile.

Animal group	Cholesterol			Triglycerides	Phospholipids
	Total	LDL-VLDL	HDL		
Basal-Control	70.2 ± 1.58	47.4 ± 1.64	22.8 ± 0.30	100.3 ± 4.92	93.7 ± 3.82
Basal-Turmeric (Raw)	71.9 ± 3.39	49.6 ± 2.30	22.3 ± 0.39	107.8 ± 7.17	95.7 ± 1.31
Basal-Red pepper (Raw)	77.3 ± 2.93	49.4 ± 1.84	27.9 ± 1.36*	99.6 ± 4.68	101.7 ± 3.27
HCD-Control	440.8 ± 14.4	429.5 ± 24.1	11.3 ± 1.14	117.1 ± 5.64	112.5 ± 6.50
HCD-Turmeric (Raw)	304.7 ± 28.6**	291.3 ± 18.2**	13.4 ± 0.91	116.1 ± 8.88	156.5 ± 9.92*
HCD-Turmeric (Cooked)	298.0 ± 38.7**	285.7 ± 20.8**	12.4 ± 0.53	110.4 ± 4.23	154.3 ± 9.39*
HCD-Red pepper (Raw)	369.4 ± 11.1**	358.2 ± 25.2**	11.2 ± 0.51	101.6 ± 5.60**	139.5 ± 6.46*
HCD-Red pepper (Cooked)	340.7 ± 29.3**	328.2 ± 28.0**	12.5 ± 0.94	98.5 ± 4.74**	150.7 ± 8.95*

Values expressed as mg/dl are mean ± SEM of 8 rats in each group.

LDL: Low density lipoprotein; HDL: High density lipoprotein; VLDL: Very low density lipoprotein; HCD: High cholesterol diet.

* Significant increase compared to corresponding control.

**Significant decrease compared to corresponding control.

Csallany (1983) using ODS column (C- 18) and an UV-Visible detector (295 nm) and a solvent system acetonitrile – methanol (1:1).

Statistical analysis

Results are expressed as mean (SEM and comparisons between groups were made by means of an unpaired Student's t-test (Snedecor and Cochran, 1976). Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Influence of dietary turmeric and red pepper on serum lipid profile in normal as well as high cholesterol fed animals is presented in Table 1. High cholesterol feeding for 8 weeks resulted in a significant increase in serum total cholesterol concentration and this increase was observed predominantly in the LDL-associated fraction. The increase in serum total cholesterol was as much as 6.25-fold. The increase in serum LDL-cholesterol is about 9-fold. Dietary turmeric and red pepper, either raw or heat processed significantly countered the extent of hypercholesterolemia. Serum total cholesterol was 31 and 32% lower as a result of feeding raw and heat processed turmeric. The same was lower by 16 and 23% in animal groups fed raw red pepper and heat processed red pepper. The reduction in blood cholesterol brought about by these two dietary spices was predominantly in the LDL-cholesterol fraction. About 32% decrease in LDL-cholesterol was evidenced in turmeric fed animals, whereas dietary red pepper produced 17 and 24% decrease in the same. The HDL-cholesterol fraction essentially remained unchanged as a result of treatment with turmeric or red pepper.

Hypercholesterolemic rats registered a 17% increase in serum triglyceride concentration (Table 1). While dietary turmeric, either raw or heat processed did not affect the serum triglyceride content in these hypercholesterolemic

rats, dietary red pepper, both raw and heat processed fully countered the increase in serum triglyceride content. Serum phospholipid concentration was 20% higher in hypercholesterolemic rats compared to basal control. Dietary turmeric, both raw and heat processed produced further increase in serum phospholipid concentration (38 - 39%) in hypercholesterolemic rats. Similarly both raw and heat processed red pepper feeding increased serum phospholipid concentration by 24 and 34% respectively.

Thus, there was practically no difference between raw and heat processed turmeric with regard to beneficial influence on serum cholesterol concentration in hypercholesterolemic rats. Even the influence of raw and heat processed turmeric on serum phospholipid content was also similar in hypercholesterolemic animals. The beneficial effect of red pepper on serum cholesterol concentration in hypercholesterolemic rats was even better in the case of heat processed spice when compared to the effect produced by raw red pepper. Similarly, the influence of heat processed red pepper on the serum phospholipid concentration in hypercholesterolemic rats was higher than that of corresponding raw spice. Dietary turmeric or red pepper generally did not have any influence on serum lipid profile in basal rats expect for a small increase in HDL-cholesterol by raw red pepper.

Liver lipid profile of normal and hypercholesterolemic rats as influenced by dietary turmeric and red pepper is presented in Table 2. Liver cholesterol was increased by 14.6-fold as a result of high cholesterol feeding for 8 weeks. This increase in hepatic cholesterol was moderately countered by dietary red pepper, either raw or heat processed by 17 and 20% respectively. Dietary raw red pepper also decreased hepatic cholesterol (by 14%) in basal rats. Dietary turmeric did not influence hepatic cholesterol level. Hepatic triglyceride concentration was elevated by 108% as a result of feeding the cholesterol enriched diet for 8 weeks. Both dietary turmeric as well as red pepper significantly countered the increase in hepatic triglyceride level in hypercholesterolemic rats.

Table 2. Influence of dietary turmeric and red pepper on hepatic lipid profile.

Animal group	Total lipids	Cholesterol	Triglycerides	Phospholipids
Basal-Control	51.1 ± 1.89	4.92 ± 0.11	25.4 ± 1.74	21.2 ± 1.66
Basal-Turmeric (Raw)	47.2 ± 0.96	5.07 ± 0.29	27.7 ± 1.19	23.8 ± 1.87
Basal-Red pepper (Raw)	44.1 ± 1.51**	4.24 ± 0.19**	18.5 ± 1.26**	20.6 ± 1.65
HCD-Control	138.3 ± 6.24	72.3 ± 2.78	52.8 ± 2.71	25.8 ± 1.63
HCD-Turmeric (Raw)	147.3 ± 5.71	71.8 ± 2.57	32.1 ± 1.37**	22.9 ± 1.88
HCD-Turmeric (Cooked)	153.4 ± 7.41	69.6 ± 3.41	27.9 ± 1.44**	23.6 ± 1.41
HCD-Red pepper (Raw)	135.9 ± 5.97	60.2 ± 2.98**	32.1 ± 1.41**	23.3 ± 0.89
HCD-Red pepper (Cooked)	147.9 ± 4.68	57.6 ± 3.20**	29.8 ± 2.99**	25.7 ± 1.61

Values expressed as mg/g liver are mean ± SEM of 8 rats in each group.

HCD: High cholesterol diet

**Significant decrease compared to corresponding control.

Table 3. Influence of dietary turmeric and red pepper on serum antioxidant molecules and lipid peroxides.

Animal group	Total thiols (mmole/dl)	Ascorbic acid peroxides (mg/dl)	α-Tocopherol (µg/dl)	Lipid (µmole/dl)
Basal-Control	17.3 ± 1.16	6.23 ± 0.20	0.783 ± 0.027	1.24 ± 0.024
Basal-Turmeric (Raw)	19.8 ± 0.73*	5.56 ± 0.27	0.852 ± 0.029	1.11 ± 0.039
Basal-Red pepper (Raw)	19.7 ± 1.19*	5.32 ± 0.09	0.800 ± 0.032	1.20 ± 0.033
HCD-Control	16.0 ± 1.30	5.20 ± 0.32	0.528 ± 0.037	1.01 ± 0.009
HCD-Turmeric (Raw)	20.0 ± 1.02*	5.22 ± 0.25	0.956 ± 0.050*	0.973 ± 0.008
HCD-Turmeric (Cooked)	14.6 ± 0.58	4.96 ± 0.22	1.127 ± 0.084*	0.978 ± 0.026
HCD-Red pepper (Raw)	20.8 ± 0.84*	5.89 ± 0.35	0.965 ± 0.025*	1.11 ± 0.019
HCD-Red pepper (Cooked)	19.7 ± 0.94	4.93 ± 0.08	1.001 ± 0.050*	0.928 ± 0.047

Values are expressed as mean ± SEM of 8 rats in each group.

HCD: High cholesterol diet * Significant increase compared to corresponding control.

The decrease in hepatic triglyceride concentration evidenced was 39 and 47% by raw and heat processed turmeric respectively, while it was 39 and 44% in the case of raw and heat processed red pepper. Hepatic phospholipid concentration was not altered as a result of high cholesterol feeding, and the same was also not influenced by either of the spices. Liver total lipid was increased in high cholesterol fed rats by 171%. The same was not beneficially countered by either dietary turmeric or red pepper. Raw red pepper however decreased hepatic total lipid by about 14% in basal rats. The significant decrease in hepatic total lipid and triglyceride content caused by dietary 5% red pepper in normal rats observed in this investigation is in agreement with a similar observation reported earlier (Sambaiah and Satyanarayana, 1982).

Influence of dietary turmeric and red pepper on the concentration of various antioxidant molecules and lipid peroxides in serum is presented in Table 3. Total thiols in serum were slightly but significantly increased by raw turmeric and raw red pepper both in basal rats as well as in hypercholesterolemic rats. This effect was not seen in the case of heat processed spices. Among the antioxi-

dant molecules, while serum ascorbic acid concentration was not influenced by either of the dietary spices; α-tocopherol was significantly enhanced by both dietary turmeric and red pepper in hypercholesterolemic animals. The extent of increase in serum α-tocopherol produced by dietary raw and heat processed turmeric was 81 and 113% respectively. Serum α-tocopherol was increased by 83 and 90% in hypercholesterolemic rats by dietary raw and heat processed red pepper. Serum lipid peroxide concentration was not influenced by dietary turmeric or red pepper either in basal rats or in hypercholesterolemic rats.

Influence of dietary turmeric and red pepper on the concentration of various antioxidant molecules and lipid peroxides in liver is presented in Table 4. Hepatic lipid peroxides were significantly lower as a result of dietary turmeric and red pepper both in normal and hypercholesterolemic situation (Table 4). The decrease in hepatic lipid peroxides produced was 28 and 13% by dietary raw turmeric and red pepper respectively in normal rats. A decrease in the same by 15 and 11% raw and heat processed turmeric was seen in the case of hypercholesterolemic rats, while raw and heat processed red pep-

Table 4. Influence of dietary turmeric and red pepper on liver antioxidant molecules and lipid peroxides.

Animal group	Total thiols (mmole/mg protein)	Glutathione (µg/mg protein)	Ascorbic acid (µg/mg protein)	Lipid peroxides (nmole/mg protein)
Basal-Control	49.1 ± 1.47	0.425 ± 0.020	1.775 ± 0.062	0.652 ± 0.036
Basal-Turmeric (Raw)	58.1 ± 3.39*	0.423 ± 0.025	1.983 ± 0.101*	0.469 ± 0.020**
Basal-Red pepper (Raw)	60.0 ± 4.23*	0.434 ± 0.026	2.913 ± 0.231*	0.570 ± 0.009**
HCD-Control	47.4 ± 2.05	0.497 ± 0.023	2.620 ± 0.109	0.503 ± 0.023
HCD-Turmeric (Raw)	39.6 ± 1.30	0.487 ± 0.029	2.090 ± 0.070**	0.426 ± 0.025**
HCD-Turmeric (Cooked)	42.3 ± 0.96	0.480 ± 0.024	2.310 ± 0.087**	0.446 ± 0.010**
HCD-Red pepper (Raw)	41.5 ± 1.28	0.448 ± 0.040	1.870 ± 0.083**	0.459 ± 0.015**
HCD-Red pepper (Cooked)	45.1 ± 1.14	0.510 ± 0.014	2.170 ± 0.076**	0.448 ± 0.021**

Values are expressed as mean ± SEM of 8 rats in each group.

HCD: High cholesterol diet

* Significant increase compared to corresponding control. **Significant decrease compared to corresponding control

Table 5. Influence of dietary turmeric and red pepper on body weight gain and liver weight in hypercholesterolemic rats.

	Body weight (g)		Liver weight		
	Initial	Final	Gain	(g)	(g/100 g body)
Basal - Control	116.2 ± 1.05	195.2 ± 4.95	79.0 ± 2.00	4.98 ± 0.12	2.55 ± 0.06
Basal – Turmeric (Raw)	116.3 ± 0.98	186.2 ± 6.86	69.9 ± 2.57	5.27 ± 0.15	2.83 ± 0.08
Basal – Red pepper (Raw)	116.3 ± 0.87	172.7 ± 4.19	56.4 ± 1.36**	4.39 ± 0.10	2.54 ± 0.05
HCD - Control	124.5 ± 1.14	182.3 ± 2.85	57.8 ± 1.90	8.02 ± 0.23	4.39 ± 0.14
HCD – Turmeric (Raw)	124.6 ± 1.08	188.1 ± 4.45	63.5 ± 1.35	8.60 ± 0.25	4.61 ± 0.13
HCD – Turmeric (Cooked)	124.9 ± 0.97	182.3 ± 2.44	57.4 ± 0.76	8.44 ± 0.17	4.62 ± 0.09
HCD – Red pepper (Raw)	124.8 ± 1.03	182.3 ± 2.80	57.5 ± 0.88	7.24 ± 0.21	3.97 ± 0.15
HCD – Red pepper (Cooked)	124.9 ± 1.05	187.5 ± 3.71	62.6 ± 1.23	7.64 ± 0.26	4.07 ± 0.13

Values are ± SEM of 8 animals in each group.

**Significant decrease compared to corresponding control.

red pepper produced a decrease of 9 and 11% respectively.

Ascorbic acid concentration in liver was favourably influenced by dietary turmeric and red pepper in normal rats (Table 4). The extent of increase in hepatic ascorbic acid was 12 and 64% in the turmeric and red pepper diet groups. These dietary spice principles did not show any beneficial effect on liver ascorbic acid in hypercholesterolemic rats. On the other hand, there were decreases in hepatic ascorbic acid of hypercholesterolemic rats in dietary turmeric and red pepper groups. The decreases in hepatic ascorbic acid were of the order of 12 to 29% in these spice groups. Hepatic glutathione content remained unaffected by dietary turmeric and red pepper in both basal as well as in hypercholesterolemic rats. Hepatic total thiols were higher as a result of dietary turmeric and red pepper only in basal rats (Table 4). The increase in total thiols was 18 and 22% in the respective diet groups as compared to control. This is the first observation on the beneficial influence of dietary turmeric and red pepper on the antioxidant status of experimental animals with respect to antioxidant molecules and lipid per-

oxides in blood and liver, although such an effect has been documented for their active principles - curcumin and capsaicin. The beneficial influence of dietary turmeric and red pepper on the antioxidant status of experimental animals with respect to antioxidant molecules and lipid peroxides in blood and liver is practically the same irrespective of whether the spice is fed raw or after heat processing. Thus, the results of this animal study suggested that although heat processing of turmeric and red pepper by pressure cooking resulted in a considerable loss of the active principles – curcumin and capsaicin, the hypolipidemic potency or the antioxidant potency of the parent spices were not significantly compromised.

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