Status paper

Green Tea Polyphenol as Food Additive and Supplemental Factor for Disease Prevention

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ABSTRACT

Green tea polyphenol (catechin, GTP) is an important factor concerning green tea flavor. GTP is produced from green tea extract by using hot water. GTP has strong antioxidative activity and effectively suppresses the production of lipid peroxides. The utilization of GTP as food additives by using the antioxidative activity of tea polyphenol for conserving the quality of food, is growing. Furthermore, the metabolism of GTP absorbed through intestines has been studied and the role of GTP as an effective free-radical scavenger is proved *in-vivo* experiments and is noticed as a biofunctional factor to prevent the induction of cancer and cardiovascular diseases.

Keywords : green tea polyphenol, GTP, chemistry, biochemistry, chemopreventive activities food additives.

1. Introduction

In the past 20 years, utilization of green tea polyphenol (GTP), also known by another name green tea extract (GTE), has grown worldwide. The utilization of GTP in several substances such as dye, detergent, deodorant, anti-tooth-carries-agent, and antioxidative additive for foods and cosmetics has been developed. Studies have also been initiated on it as a supplemental factor having chemo-preventive effects for cancer and against cardiovascular diseases.

2. The chemistry of GTP

GTP is one group of important components in green tea leaves which has many biochemical and pharmaceutical activities. GTP is composed of four kinds of catechin, (-)-epicatechin (EC), (-)-epi-gallacatechin (EGC), (-)-epicatechin-gallate (ECg) and (-)-epigallocatechin-gallate (EGCg) as main components found in green tea leaves. In adition, (+)- Gallo-catechin (GC) and (+)- catechin (C) are its minor components. The total contents of catechin in green tea leaves are higher in *Camellia sinensis* var *assamica* than in var. *sinensis* and also higher in summer shoots than in spring shoots. The chemical structure and composition patterns of catechins are summarised in Fig. 1 and Table 1.

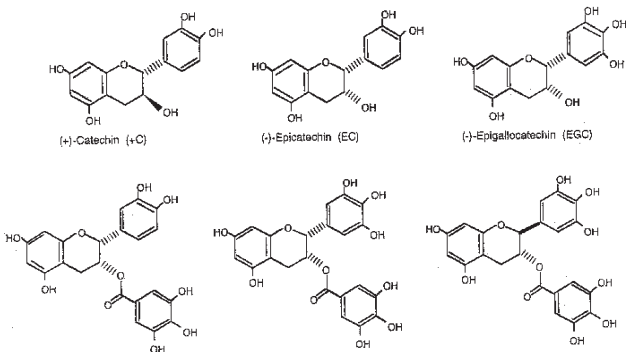

Fig.1 The chemical structures of catechins

3. The preparation of GTP

GTP is extracted from made green tea (Fig.2). Tea leaves are infused for about 20~30 times w/w of hot water at 80°~90°C for several minutes. Then, tea extract and tea waste are separated. The extract is concentrated to 20~30 % by weight of solids and spray dried. The GTP powder made by the above mentioned process is graded as GTP-30 (as Theaflavin – 30, ITOEN product) in Japan. The yield of GTP-30 averages 25% of the weight of made tea. It contains around 30% of green tea polyphenol (tea catechin). The GTP-30

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Table 1

The chemical composition of catechins in green tea leaves

A) Content of catechins in tea shoots between different varieties

	EC	EGC	ECg	EGCg
Camellia sinensis var. sinensis	1.7	3.7	2.0	6.8
Camellia sinensis var. assamica	1.5	1.7	3.1	13.1

Clone: var. *sinensis*, Yabukita, var. *assamica*, from Sri Lanka, by M. Nakagawa, 1970 May, In Kanaya

B) Variation of catechin contents between spring and summer crop in Japan.

	EC	EGC	ECg	EGC
Spring crop (May)	1.7	3.7	2.0	6.8
Summer crop (July)	1.5	3.7	3.2	11.6

Clone: Yabukita, var. *sinensis,* by M. Nakagawa, 1970, in Shizuoka

is commercially accepted by users and is fit for various uses as food additive.

The high grade GTP is prepared by a column chromatographic technique as shown in Figure 2. The GTP-30 aqueous solution is poured on the top of a resin column such as hydrophilic vinyl-copolymer, styrene-vinyl-benzene and metacrylate resin. After caffeine, amino-acids and polysaccharides in tea extract, which are not absorbed on column, are washed out by water and 30% aqueous ethanol from the column, the GTP absorbed on the top of the column is eluted out by 60% aqueous ethanol. By this process, GTP-90 containing 90% tea polyphenol and no-caffeine is prepared (Itoen, 1997). GTP-90 is useful as a supplemental factor for prevention of disorders. GTPs made by above mentioned processes, are prepared without using any organic solvents except ethanol, and are very safe with respect to the food hygiene. The chemical composition of GTPs is summarized in Table 2.

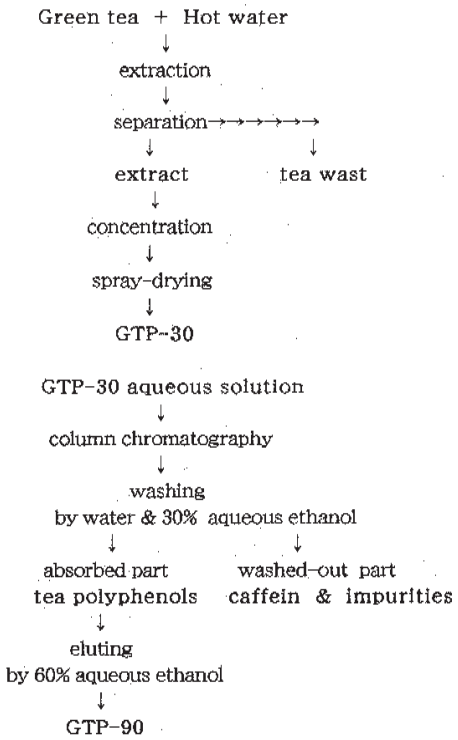

Fig. 2 The flow sheet of production process of GTPs

Table 2: Compositions of GTPs (%)

Grade	Total Polyphenol	EC	ECg	EGC	EGCg	Caffeine
30	29	2	3	9	13	4
90	93	8	16	5	59	Trace

From Theafran -30 & 90

4. GTP as Food Additives

4.1 Antioxidative action in edible oils

The lipid peroxides in food adversely affect and deteriorate the flavor of food. Various anti-oxidants have been used for keeping freshness and maintaining the quality of food. These were previously derived from natural sources such as natural tocopherol or synthetic products such as BHA. However, the use of BHA which was most popular in the past, is now suspended in several countries due to an apprehension of carcinogenicity. Also vitamin E which is considered a safe food additive, has a high cost and is inconvenient as food additive for its lipophilic property.

Water soluble GTP and a major catechin EGCg are natural and safe for consumption as food additives. They have strong and antioxidative activities for edible oil or lard as against α-tochopherol and BHA, as shown in Fig.3.

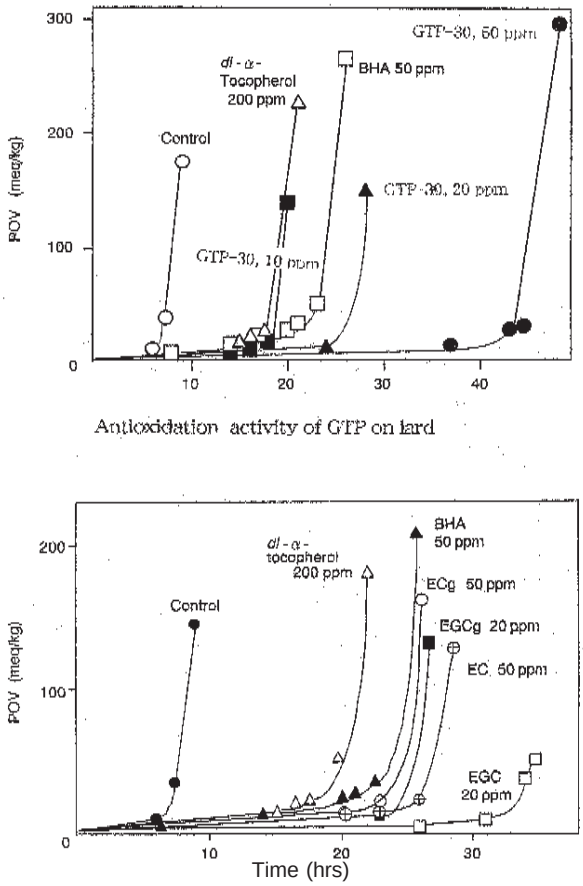

Fig. 3 Antioxidative activity of GTP and catechins on lard (AOM at 97.8°C)
(T. Matsuzaki etal., 1985

(Matsuzaki and Hara, 1985). By adding 100 ppm of EGCg to edible oil containing 600 ppm natural tocopherol, the deterioration of edible oil is reduced and its shelf-life is prolonged 2 to 3 times, but such antioxidative effect was not detected by adding 100 ppm of α tocopherol as shown in Table 3. On Japanese food market, GTP aqueous solutions have been used for keeping freshness of raw fish and protecting oxidation of unsaturated fatty acids in fish meal from deterioration, which is induced on

Table 3 Deterioration rate of edible oils (hr.)

	Salad oil	Soybean oil	Lard
Control	4	3	8
α-tochopherol*	4	3	8
EGCg*	9	9	21

Edible oils; containing 600 ppm tochopherol
* adding each 100 ppm at 120°C; air flow rate; 20 l/hr
(F. Yayabe et al. 1991)

drying and storing process. By immersing raw fish fillets in salt-water with 0.5% GTP-30, the freshness of fillets of salted or dried fish is effectively maintained and their shelf life which is usually 3-4 days, is extended up to one week.

4.2 Reduction in the loss of natural food color

The fading of carotenoid color is suppressed by addition of GTP. β-carotene aqueous solution in transparent glass bottle was completely faded within 4 days under UV light. But the decolorization of carotene was delayed for over 2 weeks by adding 0.05% GTP-90. The protective effect of GTP was larger than that of VC. (Fig. 4).

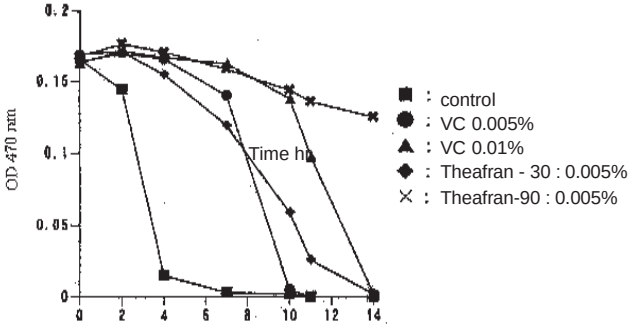

Fig. 4 The suppression effects of GTP and VC on the decolorization of β-carotene
(F. Yayanbe, 1999)

4.3 Cosmetics

UV-protecting function of GTP is utilized in cosmetics for the protection of human skin from sunburn and freckles induced by the UV-ray damage. Skin-care cream, lotion or soap containing GTP have been developed for Japanese cosmetic market.

4.4 Dental care agent

GTP or catechins showed a strong inhibitory effect on *S. mutans* which cause carries in teeth. The minimal inhibitory concentrations of EGCg for *S. mutans* is 50~100 microgram/ml. At a concentration of 20 mg/ml of GTP for 3 minutes, the number of *S. mutans* was decreased from 10^7 to 10^2. It is recommended that for foods, candies or chewing gums adding 0.1% of GTP is effective to protect tooth from carries. (Kawamura and Takeo,

1989). GTP has deodorant activity for trimethyl-amine, methylmercaptan, hydrogen-sulphide and ammonia. GTP is effective to diminish mouth odour. Mouth rinse containing 0.25% GTP has been developed (Kaneko et al, 1993).

5. Biochemical action of GTP

5.1 The metabolism of GTP in-vivo

The oral absorption and metabolism of catechins in human body have been determined during the past decade. Orally ingested catechins are distributed in intestinal mucosa, and excreted finally into feces. It was found that the ratio of catehcins taken into blood serum was less than 1% of total orally administrated dose. Most of the catechin was not absorbed in the intestine and was excreted with faeces.

The catechin concentration in the vein plasma began to increase rapidly and reached the highest level about 1 hour after the administration of GTP. Within 4 hours, catechins in plasma had disappeared. These results show that orally administrated catechins are excreted rapidly after their distribution into the tissues. (Fig. 5) (Unno and Takeo, 1995 and 1996).

Most part of absorbed catechins immediately makes conjugated compounds, as glucuronides and sulfates on the intestinal mucosa, liver and kidney. Those metabolized catechins with a small fraction of non-conjugated compounds are circulated in the human body. During the circulation in human body, glucronates and sulfates of catechin make the O-methyl 1-derivatives on the OH-radical in the β-ring and excreted with feaces as shown in Table 4 and Fig. 6 (Mao-Jung et al, 1995)

It is reported that the antioxidative activities of conjugated derivatives of catechin are about half the levels of those of catechins, and antioxidative activities of O-methyl-derivatives of conjugated catechins are very low.

5.2 Radical scavenging action

Free-radicals possessing unpaired electrons are highly reactive with other compounds. They react with lipids to produce degenerative lipid peroxides. They also denature and inactivate DNA, protein, and enzyme *in-vitro* and *in-vivo*. It is known that those activities of free-radicals induce various kinds of human diseases and aging.

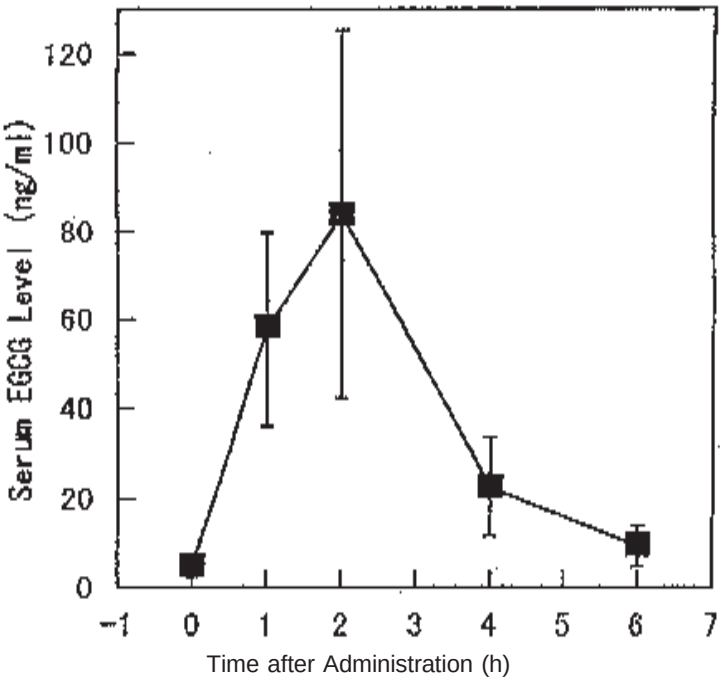

Fig. 5 Time course of plasma EGCg after its oral administration (Unno & Takeo, 1996)

Table 4 Metabolism of EC in rat (Micro M)
(I.Terao et al., 1998)

		1 hr after application		6 hr after application	
Dose	**Derivative**	**EC**	**0-MeEC**	**EC**	**0-Me-EC**
50 mg/ rat	Free	13.3	0.9	2.6	0.3
	Sulfate	0	7.6	3.6	9.3
	Glucronate	52.1	5.9	11.9	11.5
	Sulfate + Glucronate	0	23.2	5.4	28.7
	Total	65.4	37.6	23.5	49.8

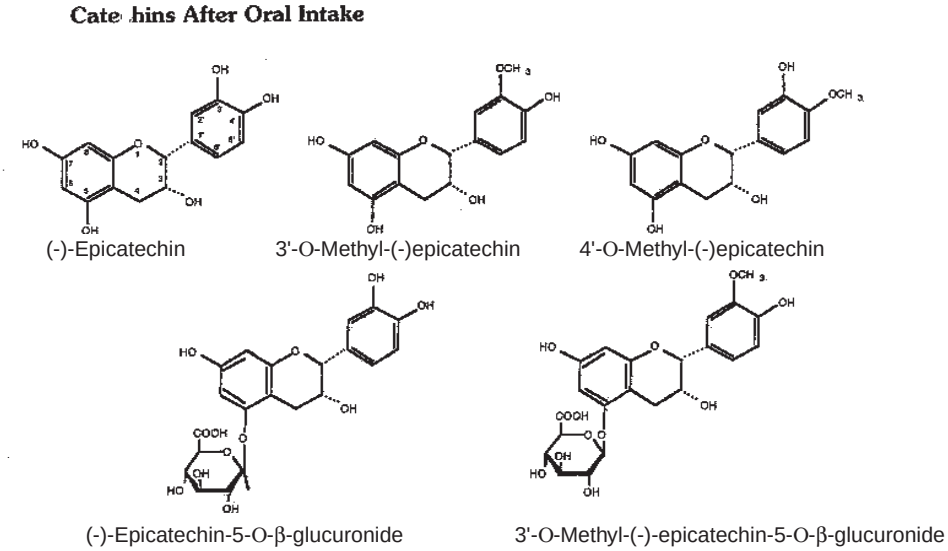

Fig. 6 Chemical structures of catechin derivatives made *in vivo* (Mao-Junglee et al, 1995)

The antioxidative role of flavanoids in food stuff as the defence system for generation of radical oxygen has been researched in great detail. Many reports on radical scavenging capacities of GTP, showed GTP to have effective radical scavenging capacities. As shown in Table 5, the superoxide anion radical (O^{2-}) scavenging power (SOD-equivalent activity) of the four dominant catechins was in the order; (-)-EGCg>(-)-EGC>(-)-ECg> (-)-EC. Those results show that pyrogallol type catechins ((-)-EGCg and (-)-EGC) were more potent radical scavengers than dihydroxyle type catechins ((-)-ECg, (-)-EC, (-)-CG, (-)-C, (+)-EC and (+)-C). It shows that the presence of a galloyl-moiety in the B-ring of catechin enhances the radical scavenging power (Unno et al, 2000).

No significant differences are observed between the scavenging capacity of tea catechins and their epimers and hence the scavenging effects of catechins are independent of their sterical structure. Those results reveal that the changes of sterical structure of catechin which are sometimes induced during the processes of tea manufacturing or GTP production, do not influence the radical scavenging activity of catechins. The (O^{2-}) scavenging activity of GTP-30 gives an SOD-equivalent value of 123±9 unit/mg of dry matter. As shown in Table 5, the sum of ($O2^{-}$) scavenging capacity of each catechin contained in GTP-30 was 106 unit/mg of dry matter. So 86% of the (O^{2-}) scavenging activity of GTP-30 can be accounted for by catechins from the calculated data. Furthermore, EGCg and EGC account for most of the (O^{2-}) scavenging activities of GTP, Table 6 (Unno et al 2002).

Table 5 Assessment of [O^{2-}] scavenging activity of samples in the PMS and NADH system (Unno et al, 2002)

Sample	IC 50* [mM]	SOD equivalent activity** [unit/micromole]
(-)-EGCg	0.36±0.01	216 ± 3
(-)-GCg	0.39 ± 0.07	205 ± 43
(-)-EGC	0.75 ± 0.08	103 ± 11
(-)-GC	0.71±0.06	109±9
(-)-ECg	0.89±0.09	88±10
(-)-Cg	1.05±0.01	74±7
(-)-EC	5.75±0.75	14±2
(-)-EC	6.06±0.52	13±1
(-)-C	4.82±0.28	16±1
(-)-C	6.08±0.50	13±1
Gallic acid	1.30±0.13	60±6
VC	0.33±0.02	237±13
Superoxide dismutase	77±5(unit/mg)	

* Concentration of sample required to reduce the relative peak height of DMPO-O^{2-} by 50% detected. Measured by ESR $V=(I \text{ control} / I \text{ test})^{-1}$

** 77 (unit / ml) IC 50 of sample (micro mole/ml)

Table 6 Contribution of catechins to the O^{2-} scavenging activity in GTP-30_9 (Unno et al, 2002)

Catechin	Composition in GTP – 30%	Measured O^{2-} Scavenging activity (unit/mg)	Actual contribution to scavenging effect of GTP-30%
(-)-EGCg	12.5	472	48
(-)-GCg	0.4	448	1
(-)-EGC	9.5	337	26
(-)-GC	1.9	356	6
(-)-ECg	2.5	199	4
(-)-Cg	Trace		
(-)-C	0.5	55	0

6. Supplemental factor for disease prevention

6.1 Cancer preventive action

Green tea has received a lot of attention as a protective agent against cancer. Several studies on green tea, black tea, oolong tea and GTP have shown inhibitory effects on oxidation and mutagenicity. Furthermore, EGCg significantly inhibited the promotion of tumours and carcinogenesis by the radical scavenger activity in animal experiments (Mackay & Blumberg, 2002). However, no clear evidence for protection effects of tea in humans has been reported. Epidemiological studies have been carried out on the preventive effects for stomach, colon and lung cancer and on the relation between tea intake and the incidence of cancer. A large number of the possibilities of the preventive effect of green tea for cancers have been reported (Kono et al, 1988, Ohno et al, 1995; Zrheng et al, 1996).

A cohort study carried out in Japan revealed that daily drinking green tea effectively prevented the initiation and promotion of cancers on humans. In 1986, the cohort study was carried out in 3,625 subjects, of the residents aged over 40 years in the town of Yoshimi in the prefecture of Saitama. In the follow-up survey after 9 years, it was recognized that the consumption of green tea was significantly associated with delaying the age of mortality by cancer. Increased consumption of green tea was associated with delaying of cancer-death-age as shown in Table 7. Especially the mortality ages of women by cancer were delayed by over 5 years over then the men who daily drink over 10 cups in a day (Nakachi et al, 1997). Those results show that GTP may prevent the cancer promotion in humans.

Recently, clinical examination has been started using GTP as a supplemental food for prevention of cancer in USA. The first phase examination has been done by using GTP capsules. After continuous study for a period of 6 months, it was shown that the maximum-tolerated dose was

Table 7 The mortality ages among human groups drinking different cups of green tea in a day (± SE. year)$_{14}$

Sex	< 3 cups	4 to 9 cups	Over 10 cups
Male	67.3±1.8(41)	69.5±1.3(66)	70.9±1.5(46)
Female	67.3±2.3(31)	70.7±104(59)	75.1±2.6(19)
Total	66.8±1.4(72)	70.1±0.9(125	71.5±1.3(65)

( ) : mortality numbers
Total examined subject numbers : 3,625
Ages of participants: over 40 years old
Period under survey: 9 years

4.2 g GTP/m^2 (1.0 g catechin) once daily or 1.0 g/m^2 GTP m^2 (0.28 g catechin) three times daily; no serious side effects on participants were observed. A dose of 1.0 g in GTP/m^2 tid is equivalent to 7~8 Japanese cups (120 ml/each) of green tea per day (Katherine et al, 2001). Now the second phase clinical examination has been started for cancer patients.

6.2 Anti-cardiovascular and anti-liver disease action

An inverse association between flavonol intake and cardio vascular and coronary heart disease (CVD) was observed in Europe. (Hertong et al, 1997). Epidemiological evidence reveals a strong inverse association between flavonol intake and CVD mortality after a 25 year follow-up of 12, 760 men (Hertong et al, 1995). Similarly, men and women from Boston area health study who consumed one or more cups of black tea per day in the previous years had a 44% lower risk of heart attack than those not drinking tea (Sess et al 1999).

The cross sectional study of effects of drinking green tea on CVD has revealed that increased consumption of green tea was associated with decreased serum concentrations of total cholesterol and tri-glyceride and an increased proportion of high density lipoprotein cholesterol, together with a decreased proportions of low and very low lipoprotein cholesterols. Furthermore, increased drinking of green tea, especially over 10 cups a day, was related to decreased concentrations of hepatological markers in serum, aspartate aminotransferase, alanine transferase and ferritin. These results show that green tea may protect against CVD and disorder of the liver (Imai and Nakachi, 1995).

It is known that GTP has an anti-hypercholesteremic effect. Dietary GTP decrease the plasma cholesterol content and increase the faecal excretion of cholesterol. It has been revealed that orally administered GTP depresses the cholesterol absorption from the intestinal wall (Ikeda et al, 1995). It was also observed that the absorbed GTP through the intestine depressed the degeneration of lipoprotein cholesterol induced by the effect of free-radicals in blood serum (Sano et al, 1995). Due to these reasons, CVD or liver diseases may be reduced by orally administrated GTP.

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