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TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

L-theanine—a unique amino acid of green tea and its relaxation effect in humans

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Since ancient times, it has been said that drinking green tea brings relaxation. The substance that is responsible for a sense of relaxation, is theanine. Theanine is a unique amino acid found almost solely in tea plants and the main component responsible for the exotic taste of 'green' tea. It was found that L-theanine administered intraperitoneally to rats reached the brain within 30 min without any metabolic change. Theanine also acts as a neurotransmitter in the brain and decreased blood pressure significantly in hypertensive rats. In general, animals always generate very weak electric pulses on the surface of the brain, called brain waves. Brain waves are classified into four types, namely α, β, δ and θ-waves, based on mental conditions. Generation of α-waves is considered to be an index of relaxation. In human volunteers, α-waves were generated on the occipital and parietal regions of the brain surface within 40 min after the oral administration of theanine (50–200 mg), signifying relaxation without causing drowsiness. With the successful industrial production of L-theanine, we are now able to supply Suntheanine™ (trade name of L-theanine) which offers a tremendous opportunity for designing foods and medical foods targeting relaxation and the reduction of stress. Taiyo Kagaku Co., Ltd, Japan won the 1998 'Food Ingredient Research Award' for development of Suntheanine™ at Food Ingredients in Europe (Frankfurt). The judges felt it was a particularly well-documented and fascinating piece of research.

The physiological and pharmacological actions of various components of green tea such as polyphenols, caffeine and γ-aminoisobutyric acid have been investigated [1–3].

L-theanine is a unique amino acid because it is found only in the tea plant, with the exception of a kind of mushroom, *Xerocomus badius*, and certain species of genus *Camellia*, *C. japonica* and *C. sasanqua*. L-theanine constitutes between 1 and 2% of the dry weight of tea leaves. It exists only in the free (non-protein) form and is the predominant amino acid component in tea, accounting for about 50% of the total free amino acids [4,5]. The occurrence of L-theanine in tea leaves was discovered in 1949 [6]. Its chemical structure was determined to be γ-ethylamino-L-glutamic acid (L-theanine) (Fig. 1). Suntheanine™ is the trade name for L-theanine and is the main component responsible for the exotic taste of green tea (known as 'umami').

Since ancient times, it has been said that drinking green tea brings relaxation. We have confirmed that Suntheanine™ can induce relaxation and improve learning ability, in addition to its characteristics of giving specific umami (a brothy or savory) taste and flavor to green tea infusion.

Our research group has established an effective method for the production of L-theanine on an industrial scale.

Physiological activity of theanine

Theanine has been known to act antagonistically against paralysis induced by caffeine. It has been revealed that theanine was absorbed quickly in the intestinal tract and showed characteristic physiological activities.

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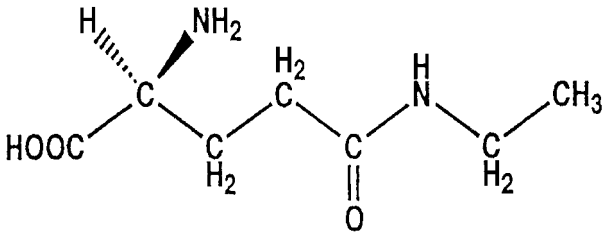

(γ-ethylamino-L-glutamic acid)

Fig. 1. Chemical structure of theanine.

Table 1. Absorption of L-theanine into the brain[a]

Time after administration (min)	Amount of L-theanine in the brain (μmol/g tissue) (% of theanine incorporated into the brain)
15	0.23 (0.06%)
30	0.39 (0.1%)

[a] Male ddy-mice weighing 18 to 20 g were used after starvation for more than 16 h. After intraperitoneal administration of 7.7 mM/kg (1.8×10^8 c.p.m./kg) of L-theanine. There was no change found in brain tissue. In the brain tissue, only L-theanine was detected on the autoradiograms.

Physiological effects of Suntheanine™

Relaxing effect

It has been shown that theanine not only gives flavor and taste to green tea but also produces a noticeable relaxation effect in human beings.

In general, animals and humans always generate a very weak electric pulse on the surface of the brain, called brain waves. Brain waves are classified into four kinds, named α, β, δ and θ waves, according to frequency. Each brain wave is related to individual mental conditions (Fig. 2). Generation of α-waves is considered as an index of relaxation. A volunteer test was performed to investigate the mental effect of theanine. Since it was expected that mental response to theanine could be varied with anxiety level, 50 female subjects (18–22 years old) were divided into two groups, namely a high-anxiety group and low-anxiety group, based on monitoring by manifest anxiety scale (MAS). Finally, the test was conducted with four high-anxiety female subjects and four low-anxiety students. Each volunteer group was given water, 50 mg theanine solution or 200 mg theanine solution once a week and brain waves were measured for 60 min after the administration. All measurements were repeated twice during a two-month test period.

In this experiment, the most remarkable α-brain waves were observed from the back to the top area of the brain surface approximately 40 min after intake of theanine solution. An oral dose of 200 mg of Suntheanine™ dissolved in 100 ml of water resulted in the generation of α-brain waves in the occipital and parietal regions of the brains of the volunteers, while only a small amount of α-waves were observed with water intake (Fig. 3). Accumulated intensity of α-brain waves showed clear tendency of dose-dependent generation of α-waves after 30 min [7] during 1 h measurement. This result is consistent with the report [8] that theanine was absorbed and reached brain tissue within 30 min of oral administration (Table 1).

It is well-known that α-brain waves are generated during the relaxed state, and it is therefore recognized that generation of α-waves is an index of relaxation. As shown in our results, Suntheanine™ can promote the generation of α-brain waves and induce a relaxed state in humans.

Lowering blood pressure

It is known that the regulation of blood pressure is highly dependent on catecholaminergic and serotonergic

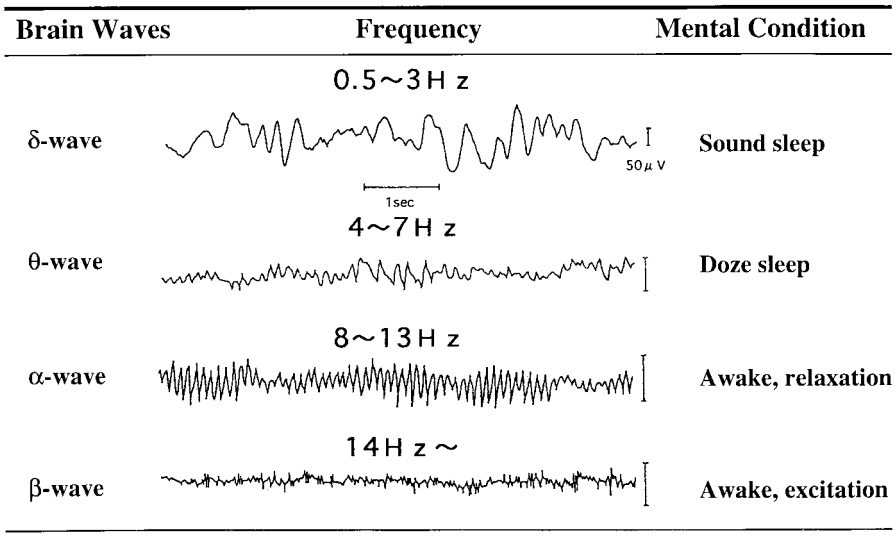

Fig. 2. Classification and relation between wave length of brain waves and mental condition.

neurons in both the brain and the peripheral nervous systems [9,10]. As it was demonstrated that theanine was absorbed and reduced serotonin levels [11,12], the lowering effect of theanine on blood pressure was investigated using spontaneously hypertensive rats (SHR). Various amounts of theanine were injected intraperitoneally to SHR and blood pressure was measured before and 60 min after administration. Glutamine was administrated as a control material. The administration of theanine resulted in a significant decrease in blood pressure. The decrease in blood pressure appeared to be dose related, and a significant decrease was observed with the administration of a high dose of theanine. However, glutamine, which is similar in chemical structure to theanine, did not exhibit an antihypertensive action on SHR (Fig. 4). This anti-

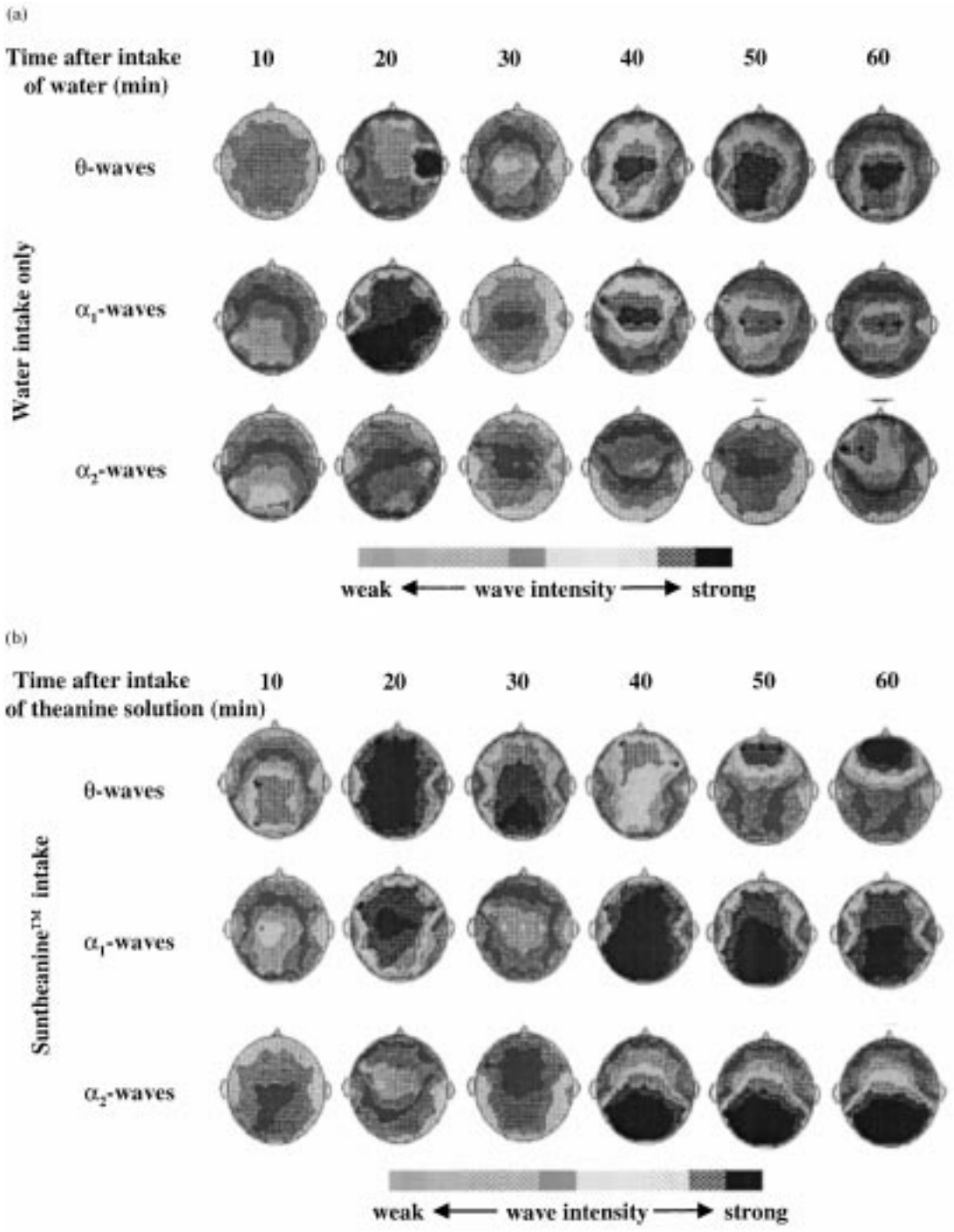

Fig. 3. (a) Topographies converted from data of brain waves on brain surface measured for 60 min after intake of water in human volunteers. (b) Topographies converted from data of brain waves on brain surface measured for 60 min after intake of Suntheanine™ in human volunteers.

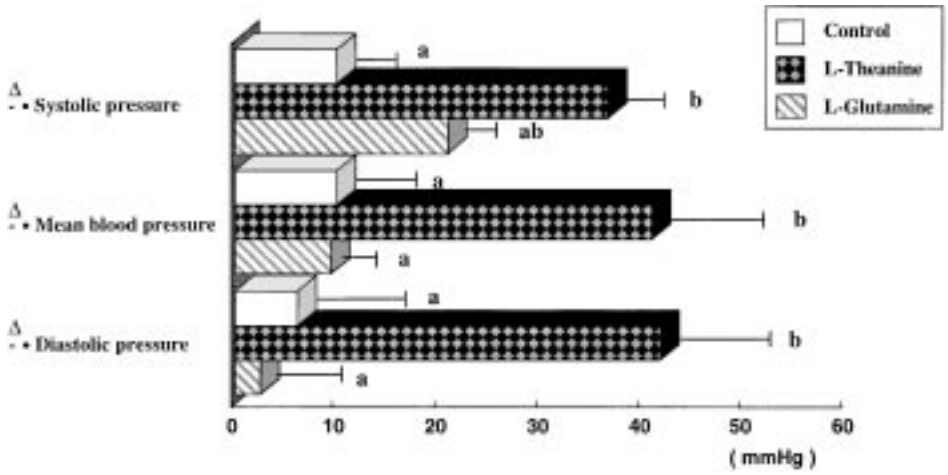

Fig. 4. Effect of theanine on the blood presure in spontaneously hypertensive rats (SHR).

hypertensive action was specific for theanine. The vascular resistance (caused by transmural pressure and blood flow), viscosity of the blood, peripheral resistance and other factors affects blood pressure generally. Theanine might affect pathways such as the peripheral nervous system and peripheral blood vessels [13,14]. Thus, theanine might have a calming effect on the mental state by lowering blood pressure.

Improvement of learning ability

It has been shown that administration of theanine has a significant effect on the release or reduction of neurotransmitters like dopamine and serotonin. It is also known that these neurotransmitters are closely related to memory and learning ability. Therefore, the effect of theanine on memory and learning ability was investigated.

Theanine (180 mg/day) was administered to weanling male Wistar rats for four months to investigate its effect on memory and learning ability. The Operant test was applied to test for memory and learning ability. Two Avoidance tests (Passive Avoidance and Active Avoidance tests) were applied to investigate memory.

The Operant test was performed under the condition that feed comes out when a lever is pushed and a light turns on. The frequency of correct responses was greater in the theanine group compared with the control group, especially with advancing sessions.

Fig. 5 shows the results of the avoidance tests. The same experiment was repeated at intervals of several hours to confirm memory. There is a general tendency that rats move to a dark place from a light place. In the ''passive avoidance'' test, an electric shock is applied soon after a rat moves from the light room to the dark. Animals that were administered theanine hesitated to move to the dark room and showed a tendency to remain in the light room longer than the control group (Fig. 5a). In the ''active avoidance'' test, the escaping behavior of rats from electric shock was examined. The

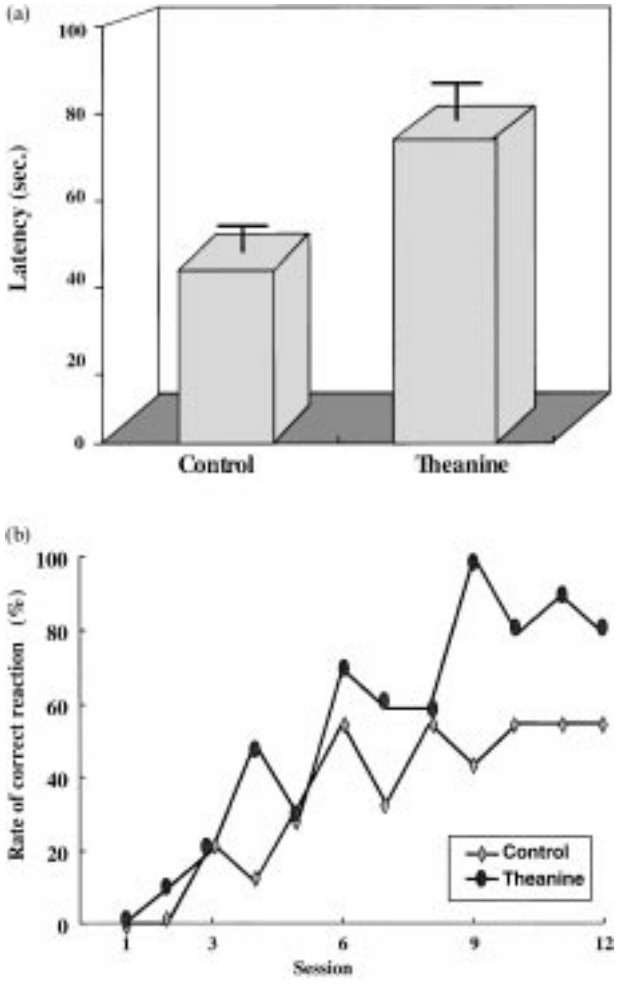

Fig. 5. (a) Average time of latency (remaining time in a light room) in rats in passive avoidance test. An electric current is passed when a rat moved from a light room to a dark room. After 24 h, the same performance is repeated. (b) Learning performance ability of rats in active avoidance test. An electric current is passed 10 s after a rat moved from a light room to the dark room to make a rat learn the danger of the dark room.

avoidance reaction rate in the theanine group was higher and increased in proportion to the number of tests (Fig. 5b). Relatively good reaction frequency in a series of memory and learning ability tests was observed in the group administered theanine solution (1 g/100 ml water) for a long period (five months).

These animal results showed the positive effects of theanine on memory and learning ability.

Absorption and metabolism of L-theanine

Intake and transportation of theanine

It has been reported that theanine intraperitoneally administered to rats was taken up by brain tissue within 30 min without any metabolic changes [8] (Table 1). It is generally known that independent transport systems exist for circulating neutral, basic and acidic amino acids. It is possible to infer that theanine may be absorbed in the intestinal tract through the methionine-carrier transport system and may show antagonism against amino acids of the same group, such as methionine, leucine, isoleucine and valine.

When theanine was administered orally to rats, it was absorbed in the intestinal brush-border membrane and incorporated into the brain via the leucine-preferring transport system of the blood-brain barrier. Theanine was transported in a dose-dependent manner into the brain. The concentrations of several neutral amino acids with large side-chains or branched-chains were decreased significantly by a high dose of theanine. On the other hand, the concentrations of alanine, serine, glycine, aspartic acid and glutamic acid were unchanged by theanine administration. Comparing the concentrations of serum amino acids, the concentrations of almost all amino acids transported via the L-system (transport system of large neutral amino acids like leucine, isoleucine, valine, etc.) were significantly decreased in the brain by the administration of theanine. This result suggested that theanine might be incorporated selectively into the brain via the L-system [15,16]. Theanine was absorbed and detected in serum and organs just after the oral administration and declined gradually over the course of 24 h (Fig. 6).

Interaction of theanine with neurotransmitters

Most Japanese people take several cups of green tea every day while taking rest, welcoming guests, or chatting with friends. Theanine is the main amino acid in green tea and may affect emotions by interacting with neurotransmitters in the brain.

The effect of theanine on striatal release of dopamine (DA) in brain was studied. The concentration of DA in striatum was significantly elevated after administration of theanine. Direct administration of theanine into brain striatum by microinjection also caused a significant increase of DA release in a dose-dependent manner [12]. As it is said that the release of dopamine, one of the brain's neurotransmitters, highly affects human emotion, these results suggest that theanine might affect the metabolism and/or the release of some neurotransmitters in the brain.

It has been reported that serotonin-containing neurons in the central nervous system participate in sympathetic control. Influence of theanine on the synthesis or degradation of brain serotonin was investigated by using an inhibitor of serotonin metabolism. Following the administration of theanine, brain tryptophan content significantly increased or tended to increase, but the contents of serotonin decreased [11,12]. These results indicated that administration of theanine might decrease serotonin synthesis and enhance the degradation or suppresses the release of serotonin.

Industrial preparation of theanine

L-theanine is synthesized naturally from glutamic acid and ethylamine in the root and transferred to the young leaves of the tea plant [17]. Many attempts have been made to produce theanine commercially. However, most of them have remained unsuccessful, because of low yields, high cost, or highly complicated processes

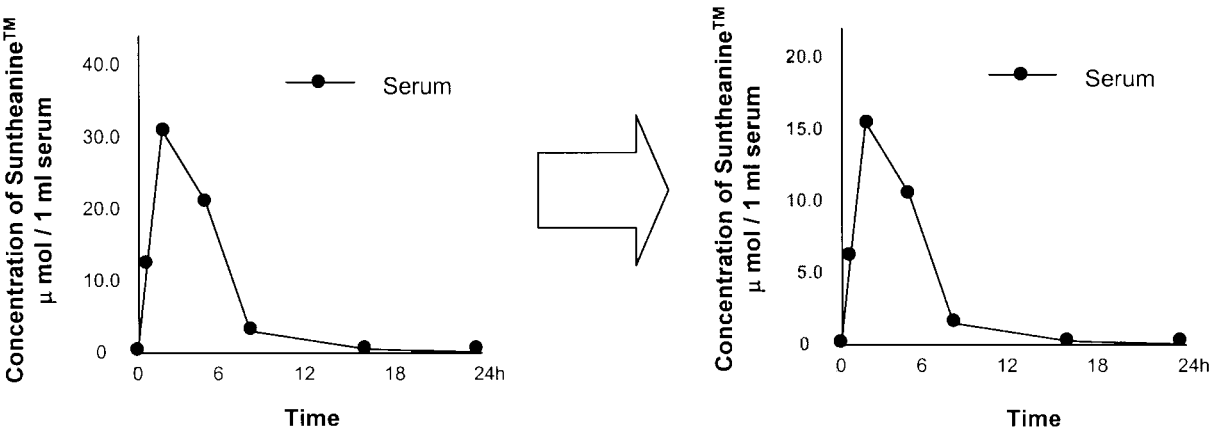

Fig. 6. Absorption and excretion of Suntheanine™ in rats.

[18–21]. We have succeeded in developing an enzymatic method to manufacture theanine on an industrial scale.

Application of Suntheanine™

L-theanine can be used for many kinds of food applications, for example, beverages, cookies, candies, ice creams and ice candies.

Stability of theanine

No degradation of L-theanine was noted in beverages heated at 121°C for 5 min. It has been shown to be stable in solution over a pH range of 3.0 to 6.6. Suntheanine™ showed good stability in both neutral (pH 6.5) and acidic (pH 3.0) beverages during 12 months' storage at under 25°C. The neutral beverage including 0.2% Suntheanine™ was autoclaved for 10 min at 90°C and the acidic beverage at 121°C for 5 min, and theanine was decomposed in the dry state at 214–215°C.

Safety aspects of Suntheanine™

The results of acute and subacute toxicity tests and a mutagenecity test performed by a public institute have confirmed the safety of Suntheanine™. There are no dietary exposure limits imposed on L-theanine by the Japan Food Additive Association. Based on L-theanine's high LD50 (5 g/kg) and the history of substantial consumption of L-theanine in green tea by a significant number of consumers over longer periods of time, no dietary exposure limits were recommended.

Applications

Suntheanine™ promotes the generation of α-waves on the brain, giving the body a sense of relaxation without inducing drowsiness. α-waves are known to indicate an awake, alert and relaxed physical and mental condition.

Suntheanine™ does not increase the level of θ-waves, and therefore does not promote drowsiness when taken in various foods. Based on the results of α-wave studies [7], we determined that administration of L-theanine between 50 and 200 mg could give relaxation effect. Suntheanine™ improves the bitter taste derived from food materials. It has been used in candies, herb tea, cocoa drinks, beverages, chocolates, puddings, jellies, chewing gums, and other confectioneries for its relaxation effect.

Conclusion

Since ancient times, it has been said that drinking green tea induces relaxation. We investigated this particular effect. It was confirmed that besides its effect of giving flavor to green tea, Suntheanine™ also had a noticeable relaxation effect.

Most people in modern times who live especially in urban areas are exposed to many kinds of mental pressures.

Suntheanine™ would be an effective way of linking food ingredients to make people feel relaxed.

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