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L-Theanine an Astounding Amino Acid in Tea, Its Synthesis and Health Benefits: A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Tea being a traditional beverage has been consumed for thousands of years becoming a daily habit that facilitates healthy living. Both black and green tea drinking have historically and generally been linked to feelings of refreshment and relaxation. Tea's L-Theanine (L-R- glutamylethylamide), a non-protein amino acid primarily found in tea leaves, is thought to be responsible for this consequence. In addition to improving tea's flavour, L-Theanine has numerous health benefits. It has a major effect on diseases linked to lifestyle, such as improving vascular function and decreasing risk of cardiovascular diseases. It also has anti- hypertensive, stress reduction, and cancer suppression activity. This amino acid can mitigate the negative effects of neurotoxins, promote normal sleep patterns, and enhance memory.

This review paper aims to explain the chemical and physical properties of L-Theanine, as well as current methods for its synthesis and extraction. It also discusses the factors that influence the theanine content of tea plants and potential health benefits that it could have for humans.

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1. INTRODUCTION

Tea (*Camellia sinensis L.*), one of the three most consumed beverages worldwide, has its origins in China. It has a complex flavour and health benefits because of the diversity of bioactive substances it contains, including theanine, catechins, and caffeine. Theanine is the most prevalent free amino acid in tea leaves, accounting for 40% to 70% of the total amount 12). It was identified in 1949 as a component of green tea, and it was isolated from gyokuro leaves in 1950 [36]. It makes up between one and two percent of the dry weight of green tea leaves [40]. Tea contains L-configuration theanine naturally, but synthesized theanines are a combination of D- and L-configuration [15]. It is also one of the most essential qualities of tea since it gives tea infusions their "umami" flavour, which promotes relaxation [14], [35].

In addition, it also offers numerous health advantages, including impacts on the immune system, urogenital protection, cardiovascular protection, cognition, memory improvement, anti-cancer and anti-anxiety properties [1], [26], [52]. This study summaries the information on tea's L-Theanine content and how it affects the scent of the beverage and its possible health advantages on human.

2. CHEMICAL, PHYSICAL, STRUCTURAL AND FLAVOUR, PROPERTIES OF L THEANINE

"L-Theanine is a non-proteinaceous transparent, colourless, odourless, water-soluble amino acid with a glutamine backbone in its core as well as an ethylamide derivative of glutamate. Although it is stable in acidic environments, glutamic acid and ethylamine are produced when the base is hydrolysed" [36]. "L-Theanine can be easily separated from caffeine, catechins, and other lipophilic tea components because it is insoluble in organic solvents like methanol and Chloroform" [51]. "Additionally, it is designated by the systematic nomenclature (2S) 2-amino- 5-(ethylamino)-5-oxopentanoic acid ($C_7H_{14}N_2O_3$, M.W. = 174.2 g/mol)" Fig 1 [36] "Theanine is a chiral molecule that primarily exists as the L(S) enantiomer in nature, similar to other amino acids, as opposed to synthesized theanine, which is often a racemic mixture of L and D enantiomers. Because of this, theanine obtained synthetically might not always have the same physiological effects as theanine found "naturally" occurring in food" [37].

"L-Theanine has been reported to cause a distinct "umami" flavour as well as sweetness in the mouth which was explained by its ability to bind to the T1R1 + T1R3 umami taste receptors" [35].

2.1 Extraction and Synthesis of L-Theanine

Various techniques to obtain L-Theanine have been developed like: Tea leaf extraction, chemical synthesis, Enzymatic & Microbiological production [2], [6].

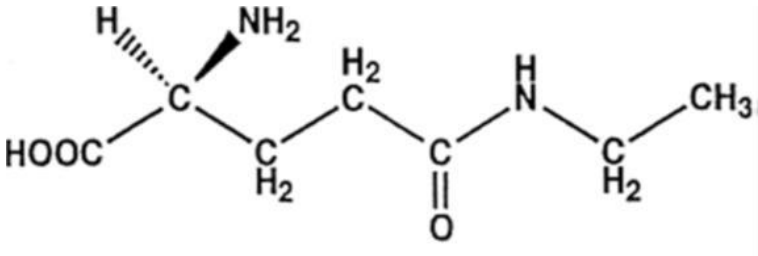

Fig. 1. Chemical structure of L-Theanine [47]**.**

3. CHEMICAL SYNTHESIS

"A more practical and affordable alternative to direct separation of the amino acid from the *Camellia sinensis* plant for large-scale production might be chemical synthesis of L-Theanine using biosynthetic techniques" [57]. "For the first time L-Theanine was chemically created by Lichenstein in 1942 by heating an aqueous solution of pyrrolidone-5-carboxylic acid to 37 degree C" [51]. "Since then, a number of additional methods for generating L-Theanine on a large scale has been devised, such as using an L-glutamine-Zn (II) complex with ethylamine was more successful (due to its high yield and reagent availability) with respect to reducing the transfer of amino acids from one peptide chain to another during the three-hour incubation at 37 degree C and subsequently leading to an increase in the L-Theanine yield. This method might appear to be the most appropriate for large-scale production of L- Theanine" [49].

"The fact that the synthetic theanine was a racemic mixture of L- and D-enantiomers rather than the pure L form found in plants was one of the main obstacles to its production and the way was less eco-friendly" [29], [48].

4. ENZYMATIC SYNTHESIS OF THEANINE

"It had been proposed that in roots of tea plants there is synthesis of theanine from glutamic acid and ethylamine and that theanine accumulation depends on the ethylamine which is derived from alanine decarboxylation catalysed by alanine decarboxylase. Due to high degradability of the enzyme commercial use of this technique in industry might not plausible" [48]. In Fig 2 Theanine synthesis from glutamic acid and ethylamine have shown.

4.1 L-Theanine Isolation from Tea

An alternate method to chemical and enzymatic synthesis for the generation of L-Theanine on an industrial scale might be the extraction of L-Theanine from tea leaves. This included ethyl acetate-based Green Tea leaf extraction, followed by preparative high performance liquid chromatography (HPLC)-based L-Theanine separation, producing an extract with about 50 g/kg L-Theanine [48]. Even though the final L-Theanine product from this process had a relatively high purity, but the higher manufacturing costs and lower overall yield made this technology less desirable to the industry [51]. Utilizing molecularly imprinted polymer (MIP) technology, L-Theanine could also be extracted. Phase inversion methods were used to create MIP compositions. After that, polymers were cleaned of any impurities using an acetic acid solution. Due to the finished product's subpar purity, the procedure was discovered to be less than viable [23], [37].

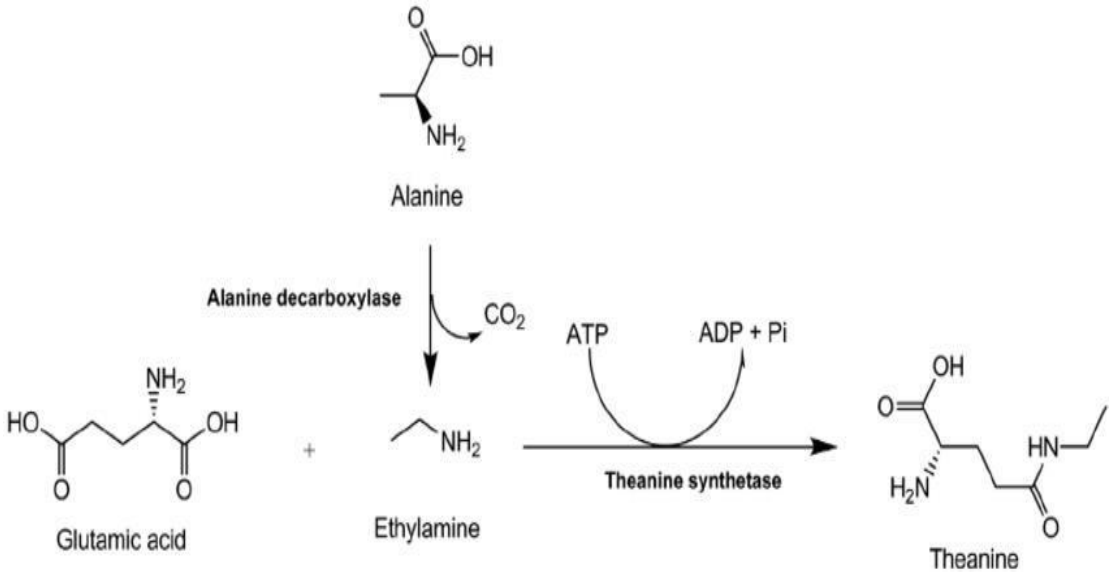

Fig. 2. Theanine synthesis from glutamic acid and ethylamine. Ethylamine formation from alanine is also shown [34]

4.2 Microbial Synthesis of L-Theanine

"Microbial production pathways for L-theanine could be broadly classified into two categories: glutamine-mediated and glutamate-mediated pathways. ATP was required to generate energy simultaneously in the glutamate-mediated process, which uses glutamate and ethylamine as precursors" [4]. Yang (2020) evaluated "a Methylovorus mays γ- Glutamylmethylamide synthetase (GMAS) and succeeded in producing 34.49 g/L of L-theanine required for optimizing both the protein expression and the reaction conditions" [54]. "By using fed-batch fermentation to introduce GMAS from *Paracoccusaminovorans*heterologously into a 5-liter bioreactor, scientists were able to reach a high level of L-Theanine synthesis of 70.6 g/L" [4].

γ-glutamyl transfer reactions with glutamine and ethylamine as precursors were used by the glutamine-mediated pathway [39]. Glutamine synthetase (GLS) and γ-glutamyltransferase (GGT) were the main catalysts in the glutamine-mediated synthesis pathway of L-Theanine, as they catalyse the conversion of glutamine and ethylamine into L-Theanine. In contrast to the glutamate-mediated synthesis pathway, ATP was not needed for this pathway. Cloning and expressing the γ-glutamyltranspeptidase from *Bacillus amyloliquefaciens* has been done in *B. subtilis* in order to perform a biosynthetic reaction using live cells [59]. Ultimately, 190mM of L-Theanine was produced by a combination of promoter screening and mutagens. The yield of L-Theanine increased significantly from 58% to 83% by using a cell-free reaction with purified enzymes and salt-tolerant mutants of the γ-glutamyltranspeptidase variant (V319A/S437G) to increase the enzyme's catalytic activity [26].

4.3 FactorsAffecting L-Theanine Content in Tea

There is variation in L-Theanine among different tea cultivars. Within the structures of the *Camellia sinensis* plant, throughout maturity, and during the growth phase, the relative concentration of L-Theanine fluctuates. According to research, L-Theanine was found throughout the entire plant at amounts ranging from 1.2 to 6.2 mg/g fresh weight, with the highest concentrations being found in the roots (6.2-13.7 mg/g). The production of L- Theanine might take place in the plant's roots and then transferred to the leaves [10].

The levels of L-Theanine vary depending on the type of tea. The average L-theanine concentration of infusions prepared from commercial tea samples quantified by high-performance liquid chromatography-diode array detector was5.13, 6.56, 6.09, and 6.26 mg/g, for oolong, black, green, and white teas respectively [3]. The accumulation mechanism of albino yellow tea was linked to the delayed catabolism of L-Theanine, and the quantity of L- Theanine in albino yellow tea was higher than that in regular green tea [7]. However, L-Theanine content in tea plants considerably varies among different cultivars and tissues. For example, the tea plant cultivars with small leaf contain higher amino acid contents than the cultivars with large leaf [21].

The expression of its metabolism-related genes was correlated with L-Theanine content. The transcription levels of CsTS2(*Camelia sinensis* L-Theanine Synthetase 2), CsGS1(*Camelia sinensis* glutamate synthetase 1), and CsGDH2(*Camelia sinensis* glutamate dehydrogenase 2) were positively connected with L-Theanine content among the 17 discovered genes related to L-Theanine metabolism, while the majority of the other genes were negatively correlated [27].

Seasonality and temperature also influence the L-Theanine content in Tea Plant. Melatonin was discovered to boost the production of L-Theanine in tea leaves at sub-high temperatures (35/30 °C) and speed up the photosynthesis of tea plants [25]. L-Theanine content in spring was shown to be substantially higher than that in summer and autumn, according to a combination of transcriptomics and metabolomics study [16].

Tea leaves from various stages such as bud, first leaf, second leaf, third leaf, and old leaf were used for research and it was discovered that the bud and first leaf had the highest concentrations of L-Theanine than matured leaves as its concentration steadily dropped [27]. In Match and Gyokuro green tea L-Theanine content rises when tea plants were purposefully shaded from sunlight [6]. Additionally, the ration of tea polyphenol to amino acid was reduced by application of nano-Se through significantly decreasing the total

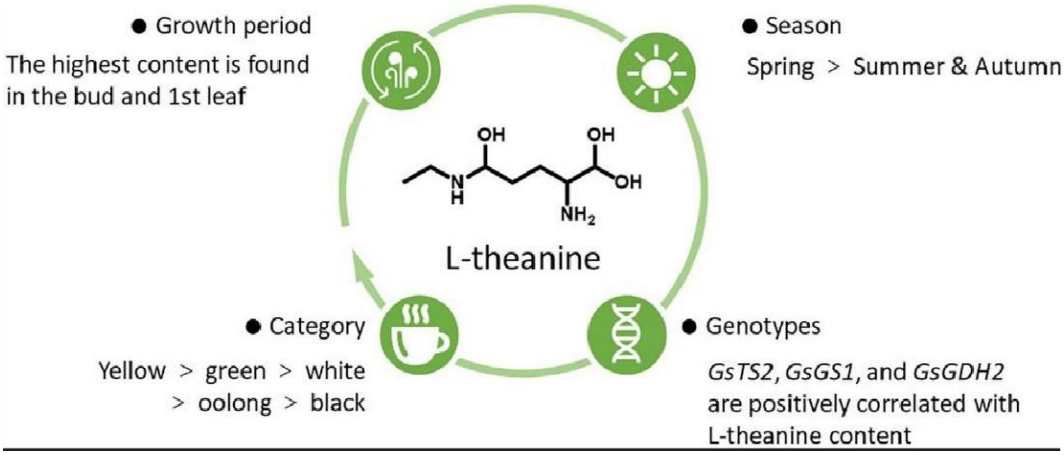

Fig. 3. Influencing factors of L-Theanine content in tea leaves [24]

amounts of catechin, caffeine, and tea polyphenols while increasing the total amounts of amino acids and theanine. Nano-Se fertilizers mainly consist of red se nano particles in very low dose with high dispersibility, low toxicity, and antibacterial properties compared to inorganic Se fertilizers [20]. Fig 3 Shows the influencing factors of L- Theanine content in Tea leaves.

5. HEALTH BENEFITS OF L-THEANINE

5.1 Anti-Tumour & Anti –Cancer Property

One of the biggest public health concerns in many countries of the world is the occurrence of cancer. Numerous studies have demonstrated theanine's anti-tumour properties both in vivo and invitro(Table:1) [28]. Theanine derivatives such as ethyl 6-nitrocoumarin-3-carboxylyl L-Theanine (TNC) and ethyl 6-fluorocoumarin-3-carboxylyl L-Theanine (TFC) showed efficientsuppression oflung cancer cell proliferation in vitro, ex vivo, and in vivo. This was done by focusing on the epidermal growth factor receptor/vascular endothelial growth factor receptor-Akt/nuclear factor-kappa B (EGFR/VEGFR-Akt/NF-kappa B) signalling pathways, which involve some of the most significant pathways in regulating the survival and proliferation of cells [58].

5.2 Improved Cognitive Learning Ability

Cognitive ability was improved by theanine through increasing the concentration of brain neurotransmitters like dopamine, 5-hydroxytryptamine (5-TH), glycine, and GABA (γ- aminobutyric acid) [53], [44]. According to a different study, consuming 47.5 mg of L-Theanine prevented extracellular glutamine from being incorporated into neurons, indicating that L-Theanine might help elderly people with cognitive impairment. Furthermore, it has been documented that L-Theaninecould control the brain's levels of serotonin and dopamine by releasing the inhibitory neurotransmitter -aminobutyric acid [51]. Table 2 shows different study results of different doses of L- theanine administration on improved cognition and learning ability.

Table 1. Anti-Tumour and Anti –Cancer Property

Study Type	Proposed Effect	References
Cancer Suppression	L-Theanine (Methyl coumarin-3-carboxylyl L-Theanine, ethyl coumarin-3-carboxylyl L-theanine, ethyl 6- fluorocoumarin-3-carboxylyl L-Theanine, and ethyl 6- nitrocoumarin-3-carboxylyl L-Theanine) showed significant inhibition of lung cancer cell migration, growth, and leukaemia in human and mouse.	[58]
Tumour Suppressor	Both separately and in combination, theanine and theobromine reduced the growth of tumours by down regulating the Akt/mTOR and JAK2/STAT3 pathways and raising the Smad2 tumour suppressor.	[41]

Table 2. Improved cognitive learning ability

Impact	Pure Theanine Treatment	References
Cognition	100.6 mg of L-Theanine showed increase in cognition among 12 men and 14 women (average age, 57.7 – 4.8 years).	**[2]**
Cognition	Sensorimotor gating was enhanced by the administration of 200–400 mg of L-Theanine**.**	[38]
Learning ability	Co-administration of 40 mg of caffeine and 97 mg of L-Theanineenhanced focus on an intersensory attention switching task**.**	[13]

5.3 Improved Sleeping Quality & Relaxation

L-Theanine showed a positive effect on betterment of mental and cognitive health by supporting healthy sleep patterns, which in turn promote better brain growth. After being tested on a specific population, the impact of theanine was found to be helpful in maintaining regular sleep patterns & getting relaxation (Table: 3) [38]. Studies showed that by enhancing slow-brain waves, controlling brain electrical activity, and raising neurotransmitter and GABA receptor levels, Mg-L-Theanine compounds enhanced the effects of L-Theanine on sleep [9].

5.4 Anti-Depressant and Stress Relief

It is commonly believed that tea (Camellia sinensis) encourages feelings of tranquillity and comfort. Tea's L-Theanine (L-ʀ-glutamylethylamide), a non-protein amino acid primarily found in tea leaves, is thought to be responsible for this consequence [50]. Table 4 shows impact of pure Theanine treatment on depression and stress related issues.

5.5 Improve Immune System

Older patients have weakened immune systems, which makes them more susceptible to influenza virus infection. Serum IgG (Immunoglobin G) and antigen-specific IgM (Immunoglobin M) levels were increased when L-Theanine and L-Cysteine were administered together. According to clinical and epidemiological research, L-theanine administration was found to prevent colds and influenza by boosting immunity and minimize the immunosuppression induced by hard activity **(Table**: 5) [5]. L-Theanine might

Table 3. Improved sleeping quality & relaxation

Impact	Pure Theanine Treatment	References
Improved sleep quality	The eight-week L-Theanine (250 mg/day) treatment of patients (17) with diagnosed schizophrenia improved the quality of their sleep.	**[38]**
Relaxation	L-Theanine (200 mg) administration promoted relaxation when the body was at rest.	**[30]**
Relaxation	Following an acute stress task, administration of L-Theanine (200 mg) led to a decrease in both heart rate and salivary immunoglobulin A (s-IgA).	**[38]**

Table 4. Anti-depressant and stress relief

Impact	Pure Theanine Treatment	References
Stress related Symptoms Reduced	Four weeks of L-Theanine administration (250mg daily) on 30 individuals (nine men and 21 women; age: 48.3 ± 11.9 years) was found to be associated with reduced stress related symptoms.	**[17]**
Depressive symptoms Reduced	Eight weeks of L-Theanine administration (250mg/day) on 20 people with major depressive disorder (open-label study) showed lower depression.	**[18]**

Table 5. Improve immune system

Impact	Pure Theanine Treatment	References
Immune function	Co-administration of L-Theanine (70 mg) and cysteine (175 mg) among 176 participants was found to be associated with a decreased risk of developing the common cold.	**[22]**
Post- operative Recovery	In patients with distal gastrectomy for cancer, co- administration of L-Theanine (280 mg) and cysteine (700 mg) during a randomised, single blind, parallel-group trial reduced post-gastrectomy inflammation.	**[33]**

mitigate heat- induced immune dysfunction by modulating the P38 signaling pathway, suppressing the rise in p-P65/P65 resulting from HSP27 (Heat Shock Protein 27) overexpression, and regulating the levels of PPAR-γ (peroxisome proliferator-activated receptor-γ) and Foxp3 (forkhead box P3) [19].

6. ANTI-OXIDANT ACTIVITY

L-Theanine demonstrated strong *in vitro* and *in vivo* antioxidant activity, according to recent studies.Due to its neuroprotective and antioxidant characteristics, L-Theanine could significantly reduce the risk of cadmium-induced brain damage in rats [43]. In another study, the administration of L-Theanine improved the striatum's antioxidant capacity by lowering the levels of lipid peroxide and nitric oxide (NO) in orofacial dyskinesia (OD) rat models induced by haloperidol (HAL) [45]**.**

7. CARDIOVASCULAR PROTECTION

The risk of cardiovascular disease waslowered and vascular function was enhanced by tea drinking through lowering blood cholesterol, increasing nitric oxide production and arterial vasodilation and shielding the brain from cerebral ischemia injury [42]. L-Theanin was found to reduce the risk of cerebrovascular disorders by lowering the serum cholesterol levels [28].

8. IMPROVEMENT OF INTESTINAL IMMUNITY

Intestinal mucositis and diarrhoea could be prevented by combining L-Theanine and cystine (5:2, w/w) as they could also prevent the reduction of (glutathione GSH) levels, inhibit ROS (Reactive Oxygen Species) production and oxidative stress, and shorten intestinal villi and destroy crypts caused by 5-FU (5-Fluorouracil) [56].

In addition to causing severe intestinal damage, radiation therapy also suppresses bone marrow. The crypt cells found in small intestines are especially vulnerable to radiation and prone to dying. L-Theanine and cysteine pre-treatment could lengthen the villus, deepen the crypt, and decrease the number of apoptotic cells in the jejunal crypt following radiation exposure [32].

9. UROGENITAL PROTECTION

L-Theanine also demonstrated both *in vitro* and *in vivo* protection of the urogenital system. L-Theanine inhibited pro-inflammatory protein kinase C (PKC)/ERK (extracellular signal-regulated kinase)/NF-kB(Nuclear factor kappa B)/intercellular adhesion molecule 1 (ICAM-1)/IL-33 (Interleukin-33) signaling, oxidative stress, apoptosis, and autophagy in female Wistar rats given urethane anaesthesia, thereby mitigating substance P-induced bladder hyper function [46]. Furthermore, research on stem cells revealed that 50 mM L-theanine might stimulate human stem cell proliferation and glucose metabolism to sustain the Krebs cycle, which was critical to avoiding interruption of spermatogenesis [11]. According to the findings, oral L-Theanine might, at least in part, prevent bladder dysfunctions by preserving bladder contractility and preventing chronic sympathetic hyperactivity [31].

10. POSSIBLE SIDE EFFECTS OF L-THEANINE

There is little information on L-Theanine used alone, and its adverse effects are poorly understood. Nonetheless, L-Theanine was categorized by the U.S. Food and Drug Administration (FDA) as a food ingredient that is generally acknowledged to be safe up to 250 milligrams per serving [40]. It was found that tea could upset the stomach, especially when taken in large amounts, therefore those who

drink it or take L-Theanine as green tea extract should be mindful of this. Furthermore, a small number of reports of liver issues had involved users of green tea extract [55]

Pregnant or nursing women are not yet safe to take L-Theanine, according to medical experts. Before taking L-Theanine, green tea extract, or drinking more tea, pregnant or nursing woman should consult with their doctors [8].

11. CONCLUSIONS

A major amino acid called L-Theanine is present in tea leaves and is responsible for the "umami" flavour. Climate and growing period have an impact on the amount of L-theanine found in different types of tea.The yields and commercial viability of the L-Theanine synthesis have varied; the majority were labour- and time-intensive with relatively low L-Theanine yields. The commercial production of purified L-Theanine appears to lack a technique that could produce the product in an environmentally and financially sustainable manner. To meet the high market demand in recent time eco-friendly microbiological techniques have been developedfor production of L-Theanine.

L-Theanine has also generated a great deal of interest as a functional food ingredient and supplement due to its therapeutic benefits, which include effects on immunological function, learning capacity, memory improvement, urogenital protection and cancer suppression. Additionally, L-Theanine stimulates the brain's production of α-waves, which relaxes the body without making it feel sleepy. However, it is also important to acknowledge that additional research is required to support any clinically meaningful claims regarding L- Theanine as a potential functional food ingredient when compared to its pure encapsulated form.

COMPETING INTERESTS DISCLAIMER

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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