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Review

L-Theanine as a Functional Food Additive: Its Role in Disease Prevention and Health Promotion

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Abstract: Tea has been consumed for thousands of years and is an integral part of people's daily routine, as an everyday drink and a therapeutic aid for health promotion. Consumption of tea has been linked to a sense of relaxation commonly associated with the content of the non-proteinogenic amino acid theanine, which is found within the tea leaves. The aim of this review article is to outline the current methods for synthesis, extraction and purification of theanine, as well as to examine its potential benefits related to human health. These include improvements in cognitive and immune function, cancer prevention, reduced cardiovascular risk and its potential usefulness as a functional food product.

Keywords: theanine; green tea; alpha wave production; relaxation; functional food, bioactives

1. Introduction

Tea is a one of the most widely consumed beverages in the world, second only to water [1]. Derived from the Theaceae family, the *Camellia sinensis* species is commonly used for the production of green tea (GT) [2]; it accounts for up to 22% of tea produced and consumed worldwide [3,4] and it is predominantly grown in Asian countries. Historically, tea consumption has been associated with relaxing effects [5]. More recently, numerous other health benefits, including but not limited to antioxidant, antimutagenic, and anticarcinogenic effects have been identified for its constituents such as the polyphenols (catechins), caffeine and other flavonoids. These compounds may account for up to 30% of the dry weight [1]. Twenty-six different amino acids have been reported and identified in GT plants [6], with the most abundant being *N*-ethyl-L-glutamine, glutamic acid and aspartic acid [7].

N-ethyl-L-glutamine, commonly referred to as L-Theanine (L-THE) [8], is most commonly found in GT leaves, although it has also been identified in the basidiomycete mushroom (*Xerocomus badius*), at much lower concentrations [8]. In the late 1940s, L-THE was isolated as an extract from an aqueous solution of dried tea leaves [9]; however, L-THE was first chemically synthesised from pyrrolidonecarboxylic acid and aqueous ethylamine [10].

Currently, there is substantial interest in L-THE and several potential health benefits have been attributed to it. This narrative review will focus on the chemical and physical properties of L-THE, and its impact on human health in general, including but not limited to its usefulness as a functional food additive.

2. L-THE in Nature

The relative concentration of L-THE in the *Camellia sinensis* plant varies between the plant's structures, over maturation and through the growth period. Recent studies have indicated that L-THE is distributed across the entirety of the plant with concentrations ranging between 1.2 and 6.2 mg/g fresh weight, with higher concentrations being expressed in the roots (6.2–13.7 mg/g). The biosynthesis of L-THE is proposed to occur in the roots of the plant and it is then transported towards the leaves [11]. The shading treatment and nitrogen fertilisation have been shown to influence the L-THE levels and the total free amino acid content in the *Camellia sinensis* plant [12]. Although most often related to GT, L-THE is present at similar levels in other types of teas made from the plant, including black, white and oolong teas [13].

3. Chemical, Physical and Flavour Properties

L-THE is a water soluble non-proteinous amino acid [14] containing a glutamine backbone within the core of L-THE as well as existing as an ethylamide derivate of glutamate [15]. It is stable in acidic conditions but yields glutamic acid and ethylamine during base hydrolysis [8,16]. L-THE is insoluble in organic solvents such as chloroform and methanol, which facilitates the easy separation of L-THE from caffeine, catechins and other lipophilic tea constituents [8]. Aqueous L-THE solutions (1% (w/v)) stabilised at pH 5–6, were found to be stable (>1 year) under normal environmental conditions [8,16,17] and have a boiling point higher than that of water (range 214–216 °C) [8]. Furthermore, its systematic nomenclature is described as (2*S*)-2-amino-5-(ethylamino)-5-oxopentanoic acid ($C_7H_{14}N_2O_3$, M.W. = 174.2 g/mol) [18]. Gamma-glutamylethylamide [18] and L-glutamic acid-gamma-ethylamide have also been used to denote L-THE and it is also available under the proprietary name Suntheanine® [5,19]. Similar to other amino acids in nature, theanine is a chiral species and occurs predominantly as the L-(*S*) enantiomer (Figure 1), whereas a synthetically derived theanine is typically a racemic mix of L- and D-enantiomers. As such, the use of synthetically derived theanine may not necessarily exhibit the same physiological effects as theanine found "naturally" occurring in foods [8].

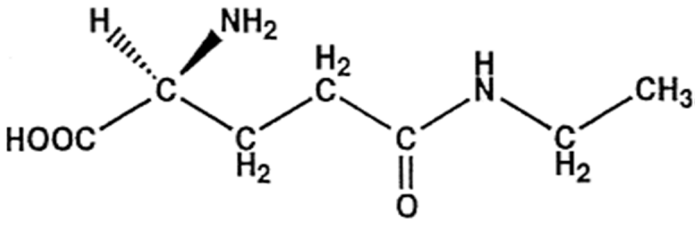

Figure 1. Chemical structure of L-THE. Adapted from [5].

L-THE is reported to induce sweetness along with a unique "*umami*" taste on the palate [5]. This is attributed to its capacity to bind with the T1R1 + T1R3 umami taste receptors and giving a similar taste sensation to that of monosodium glutamate [5,20,21].

4. Extraction and Synthesis of L-THE

L-THE can be obtained in three ways: extraction from tea leaves, chemical synthesis or biosynthesis.

4.1. Isolation of L-THE from Tea

Isolating L-THE from tea leaves has been proposed as an alternative method for industrial scale L-THE production compared to chemical and enzymatic synthesis. This includes, extraction of GT leaves using ethyl acetate followed by isolation of L-THE using preparative high performance liquid chromatography (HPLC), yielding an extract containing around 500 g/kg L-THE [8]. However,

even though this procedure provides a final L-THE product with relatively high purity, excessive production cost and lower overall yield makes this methodology less appealing to the industry [22].

L-THE can also be isolated using molecularly imprinted polymer (MIP) technology. MIP polymer formulations are prepared using phase inversion techniques. Polymers are then washed with acetic acid solution to remove any impurities. However, the method was found to be less than viable due to the mediocre purity of the final product [8,23].

4.2. Chemical Synthesis

Chemical synthesis of L-THE, using biosynthetic methods, offers a more convenient and cost-effective alternative for large scale production compared to direct isolation of the amino acid from the *Camellia sinensis* plant [24]. L-THE was first chemically synthesised by Lichenstein in 1942 by treating pyrrolidone-5-carboxylic acid with an aqueous solution of ethylamine at 37 °C [10]. Since then, several other approaches have been developed to chemically synthesise L-THE for large scale production, such as utilising γ-benzyl glutamate dissolved in pyridine trityl chloride with the addition of ethylamine, producing up to 340 g/kg [25]. Similarly, a two-step method was reported that involved the dehydration of L-glutamic acid to pyrolidone carboxylic acid (pyroglutamic acid), followed by reaction with ethylamine yielding up to 375 g/kg of L-THE [26]. More recently, L-THE was synthesised from N-phthaloyl-L-glutamic acid by treating with acetic anhydride, followed by ethylamine. Subsequent incorporation of the hydrazine hydrate to the above reaction, enabled breakdown of several amine units, producing theanine with a 700 g/kg overall yield [27]. Although this particular method would seem to be the most appropriate for large scale manufacturing of L-THE, using a L-glutamine-Zn(II) complex with ethylamine is 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 °C and consequently leading to a 17% increase in the L-THE yield [28]. However, this type of chemical synthesis is less environmentally friendly as it generates chemical waste compounds that can have negative effects on the environment, making it less appealing for the consumer [8].

One of the main limitations found with the production of synthetic theanine is that the synthetic product can be a racemic mixture of L- and D-enantiomers (due to a change in the chiral system) as opposed to the pure L form found in the plants, leading to considerable uncertainties regarding its safety and efficacy. Furthermore, the time and costs associated with L-THE synthesis varies considerably between methods, especially those that require protection and de-blocking procedures for its reactive groups [8].

4.3. Enzymatic Synthesis of Theanine

Theanine is produced in all organs of tea seedlings with the roots as the major site of L-THE biosynthesis in adult tea plants. In plants, L-THE is synthesized from glutamic acid and ethylamine in the presence of the enzyme *theanine synthetase*. However, this particular enzyme is easily degraded and therefore, its use in commercial manufacturing processes is not plausible [8].

Other techniques for the formulation of L-THE for commercial purposes have been devised using bacterial enzymes such as *γ-glutamyltranspeptidase*, *glutamine synthetase* and *glutaminase*. Some studies have reported the use of a *glutaminase* derived from *Pseudomonas nitroreducens* IFO 12 694 to hydrolyse glutamine to glutamic acid, which then reacts with ethylamine to produce L-THE. However, using glutamine as a starting material is preferred to using glutamic acid as this is more expensive and time consuming and the acid is less stable than glutamine [8,29,30]. Other noted methods include the synthesis of L-THE from glutamic acid and ethylamine using a glutamine synthetase from *Pseudomonas taetrolens* Y-30, involving sugar fermentation from baker's yeast cells [31], or using a *γ-glutamylmethylamide synthetase* from *Methylovorus mays* and a *glutamine synthetase* from *Bacillus subtilis* [32,33]. Other successful non-ATP dependent methods use a *γ-glutamyltranspeptidase* derived from *E. coli* k-12 derivatives and *Bacillus licheniformis* ER-15 with glutamine as the starting

substrate; however, some require a high concentration of ethylamine to induce the conversion of glutamine to theanine [34–36]. Although current methods are relatively complex, biosynthesis of L-THE shows strong potential for an industrial-scale manufacturing method as the current enzymes used in the preparation methods offer the advantage of producing theanine in its naturally occurring L-forms [8,16].

5. Analytical Methods for the Determination of L-THE Levels

A number of techniques have been developed that are useful for determining the concentration, yield and purity of L-THE. The total content of free amino acids in tea infusions can be determined using ninhydrin-based Fourier transform near-infrared spectroscopy (NIR) and ninhydrin or 2,4-dinitrofluorobenzene derivatisations [7,37]. Recent findings have suggested that this technique provides a rapid quantifiable prediction of total amino acid content in tea leaves. Individual analysis of free amino acids can be subsequently determined by using techniques such as high performance liquid chromatography (HPLC) accompanied with diode array detectors (DAD) or anion exchange chromatography and electrokinetic capillary chromatography (ECC) [38–40]. Similarly, a 100% success rate has been reported for differentiation of amino acids (alanine, arginine, asparagine, aspartic acid, glutamic acid, isoleucine, histidine, leucine, phenylalanine, serine, theanine, threonine, and tyrosine) in different teas (e.g., green, black, Oolong, white, and Pu-erh teas) by liquid chromatography using derivatisation with *o*-phthalaldehyde and fluorescence detection methods [41].

Anion exchange chromatography separates negatively charged amino acids across a slide medium without derivatisation [42,43]. With L-THE lacking a chromophore group, the use of chromatographic methods for its estimation requires pre or post-column derivatisation to allow high sensitivity for ultraviolet (UV), visible, fluorometric, or electrochemical detection. However, such methods generally require expensive instrumentation and reagents [44]. On the other hand, ECC involves the application of different voltages across buffer filled capillaries to separate colloids based upon their differences in electrophoretic mobility. Due to the high-speed analysis and efficiency (less than 8 min of quantitative chromatography) [45], ECC presents itself as a strong alternative if not a complementary technique to HPLC to analyse free amino acids, including L-THE, and other tea constituents [40].

A relatively simple colorimetric assay using enzymes (an environmentally friendly and low cost approach) has also been reported. It involves the coupled reaction of *Pseudomonas nitroreducens* NBRC 12694 (PnGGT, involved in catalysing theanine synthesis from glutamine and ethylamine effectively by a transfer reaction) and an amine dehydrogenase from *Paracoccus denitrificans* NBRC 12442 (PdADH, involved in catalysing the oxidative deamination of primary amines as well as aromatic amines, and shows a strict substrate specificity toward amines) [44]. The micellar electrokinetic capillary chromatography technique has also been reported for quantification of L-THE. It has shown to be a leading method for determining free amino acid concentrations from solutes with the use of UV and DAD detectors. This technique combined electrophoretic and chromatographic isolation principles, where surfactants were introduced to the buffering solution to form micelles and lead to free amino acid separation based on size and charge [39,46].

6. Pharmacokinetic Properties of L-THE

There are limited studies that report on the absorption and pharmacokinetics related to total L-THE consumption in human trials. Human kinetic data from a study by Scheid *et al*. [47] showed that L-THE is absorbed from the intestinal tract into the systemic circulation within 10–24 min followed by its transportation into a range of tissues, including the brain. In the same study, it was reported that the maximal plasma concentrations of L-THE occurred 0.8 h after capsule (100 mg) and tea administration, which contained the same amount of L-THE [47]. It was also suggested that L-THE is metabolised to ethylamine and glutamic acid, as their respective concentrations in blood plasma increased in the subjects, and that it was excreted in the urine with some retention in erythrocytes [47]. Similarly, a relatively recent study by van der Pijl *et al.* [48] investigated the absorption of L-THE in

enriched teas and of biosynthetic L-THE at varying doses (25–100 mg). The maximal blood plasma L-THE levels reached 1.0–4.4 mg/L, 50 min after consumption [48]. Based on these human trials, it is evident that the L-THE reaches a maximal concentration in the blood between 30 and 50 min after oral administration [5,8]. Currently, there is no indication that excessive intake of L-THE produces major side effects in humans. The US Food and Drug Administration (FDA) recommends that a total daily consumption should not exceed 1200 mg; however, toxicity studies must be undertaken to fully understand the long-term side effects of L-THE supplementation in humans [8].

7. Animal Toxicity Studies

Several studies examining L-THE toxicity in animal models have been reported. One study conducted a 13-week dietary toxicity and toxicokinetic studies with L-THE in male and female rats [49]. A maximal quantity of 4000 mg/kg weight/day was administered through dietary integration. The study revealed no consistent, statistically significant treatment-related adverse effects on behaviour, morbidity, mortality, body weight, food consumption and efficiency, clinical chemistry, haematology, urinalysis, gross pathology, organ weights or ratios or histopathology. It was concluded that there were no significant adverse effects in the animals for any of the parameters investigated at the maximal dosage tested [49].

In another study in rats, repeated administration of L-THE, 10 mmol/kg body weight/day through gastric intubation for two weeks, caused a decrease in the peroxidation levels of lipids within the brain, which could be a positive effect. More importantly, it was shown that repeated administration was associated with increasing protein expression levels of the gene coding protein PLC-β1, which is important for intracellular signalling of the cortex in the brain [50]. The cytotoxic effects of L-THE on cultured rat hepatocytes has also been examined, where a maximum quantity of 3 mg/mL of L-THE provided no indication of cytotoxic effects [51]. Theanine administration has been associated with a significant decrease in neutral amino acid concentration, especially those with large side-chains or branched-chains, but the relevance of this is unknown. In contrast, the concentrations of alanine, serine, glycine, aspartic acid and glutamic acid were unchanged in the presence of L-THE [5].

8. L-THE and the Brain

8.1. Production of Alpha Waves in Brain

The consumption of L-THE has been reported to have a number of modulatory effects on brain function, including the generation of electric pulses on the brain surface called brain waves (Table 1) [8]. Classified into four types α, β, δ and θ waves, these waves potentially act as indicators of continuous electrical activity inside the brain as determined by electroencephalogram (EEG), which effectively measures electrical activity/impulses through electrodes attached to the scalp [5]. Several studies have shown that L-THE intake (50–200 mg) significantly increases the pattern intensity of α-wave production in the different areas of the cerebral cortex without causing drowsiness due to unchanged θ-waves [5,8,52]. An increase in α-waves in the cerebral cortex has been proposed as an index of an increased relaxed but alert mental state [8,53].

In addition, several studies report that the administration of 250 mg and 400 mg L-THE had profound modulatory effects that resulted in improved sleep quality in animal models and in human trials, including one study in individuals with schizophrenia [54–56]. In addition, L-THE (200 mg) has been reported to promote a reduction in resting heart rate, which further highlights its relaxing properties [57].

Table 1. The effect of pure L-THE consumption on generation of wave production and its patterns in the human brain.

Impact on	Pure Theanine Treatment	Reference
α-Wave production	Administration of L-THE (50–200 mg) in 6 female participants displayed an increase in alpha wave production observed 40 min after oral ingestion. This presented a relaxing effect without causing drowsiness due to unchanged θ-waves.	[5]
α-Wave production	Ingestion of L-THE (50 mg) enhanced α-wave production in young participants.	[19]
α-Wave production	Administration of L-THE (200 mg) in 8 females enhanced the generation of α-wave production.	[52]
Relaxation	Administration of L-THE (200 mg) may increase relaxation under resting conditions.	[58]
Relaxation	Administration of L-THE (200 mg) resulted in a reduction in heart rate and salivary immunoglobulin A (s-IgA) in response to an acute stress task.	[57]
Improved sleep quality	Administration of L-THE (400 mg) may improve sleep quality in boys diagnosed with ADHD.	[54]
Improved sleep quality	Treatment of diagnosed schizophrenia patients (8 weeks) with L-THE (250 mg) was effective in improving sleep quality.	[56]

8.2. Effect of L-THE on Cognition and Learning Ability

Numerous studies have highlighted the effects of L-THE on learning ability and on a multitude of neuroprotective effects (Table 2) [8]. One specific study, involving administration of L-THE (200 mg/100 mL water), reported profound effects on attention performance as well as on reaction time responses in normal healthy subjects prone to high anxiety [59]. There is also evidence to support its synergistic relationship with caffeine. Haskell *et al.* [60] conducted a regimen of tests prior to and after combined consumption of L-THE (250 mg) and caffeine (150 mg) and found significant cognitive improvements in numeracy, sentence verification and overall alertness. Furthermore, compared to a placebo, there was an increase in the speed and accuracy of performance in an attention-switching task following the ingestion of a combined 100 mg L-THE and 50 mg caffeine [61]. Likewise, improvements in cognitive performance, subject alertness and complex senses interactions have also been documented [62–64]. On the other hand, findings from Ritsner and colleagues found no evidence that L-THE (400 mg/day) improved cognitive function, attention or learning [65]. However, these studies were performed on schizophrenic patients compared to healthy subjects examined in other reported studies. Current evidence also suggests that L-THE is a useful "add on" to the prophylactic management of schizophrenia using antipsychotic treatments, as L-THE can augment the effect of antipsychotic therapy by ameliorating positive activation and anxiety symptoms in schizophrenia and schizoaffective disorder patients [65].

Another study found that ingestion of L-THE (47.5 mg) inhibited incorporation of extracellular glutamine into neurons suggesting that L-THE may improve cognitive dysfunction in elderly [66]. Similarly, administration of 200 mg L-THE was found to have an "*anti-stress*" effect on pharmacy students [67]. Moreover, L-THE has been reported to regulate dopamine and serotonin levels in the brain through the release of the inhibitory neurotransmitter γ-aminobutyric acid [68]. It is also known that L-THE is a good substrate for the glutamine transporters [69] and therefore, it could play a role similar to glutamine in brain physiology and function. These findings suggest that L-THE elicits a relaxing effect on the brain, provides many neuroprotective roles, and also has a profound impact on learning ability and cognition.

Table 2. The effect of L-THE consumption on learning and cognition.

Impact on	Proposed Effect	Reference
Learning ability	Co-treatment of L-THE (250 mg) and caffeine (150 mg) enhanced reaction time, working memory and sentence verification accuracy.	[60]
Learning ability	Intake of a combination of L-THE (97 mg) and caffeine (40 mg) improved attention on an inter-sensory attention switch task.	[61]
Learning ability	Administration of L-THE (250 mg) enhanced α-wave activity over the parieto-occipital scalp during the inter-sensory attentional cuing task.	[53]
Learning ability	Co-administration of L-THE (100 mg) and caffeine (50 mg) increased speed and accuracy of performance of an attention-switching task.	[70]
Cognition	Co-administration of L-THE (100 mg) and caffeine (50 mg) enhanced tonic apportionment of attentional resources to visuospatial attentional deployment.	[62]
Cognition	Co-intake of L-THE (97 mg) and caffeine (40 mg) improved cognitive performance and increased subjective alertness in young adults.	[63]
Cognition	Administration of L-THE (200–400 mg) increased sensorimotor gating.	[64]
Memory loss	Ingestion of L-THE (47.5 mg) showed a lower decline in cognitive function in elderly patients.	[66]

9. Systemic Effects of L-THE

Immune System

The body's defence against foreign molecules relies on the homeostatic regulation of innate and adaptive immune systems [71]. Conventional methods of treatment for common illnesses usually involve a regimen of antibiotics, antivirals, immunosuppressant's and dietary intervention that may support or enhance immune function [72]. A number of recent studies have suggested that L-THE administration can improve the body's immune system. The key findings have been summarised in Table 3 [72,73]. One particular study highlighted the use of L-THE as an intervention to decrease the incidence of upper respiratory tract infection symptoms via enhancing γ and δ T lymphocyte function [74]. In particular, in elderly patients displaying low serum protein, a supplementation regimen with L-THE (280 mg) and cysteine (700 mg) enhanced the primary antibody response to the influenza vaccine in elderly participants [74,75].

Another study found a link between the synergistic effect of L-THE and cysteine in ameliorating inflammatory responses in the intestinal tract [76]. Miyachi *et al.* [76] demonstrated that the co-treatment of L-THE (280 mg) and cysteine (700 mg) post-surgery alleviated post-gastrectomy inflammation. This particular study highlighted the potential use of L-THE in the perioperative period through the achievement of a stable postoperative course and early recovery rates [76]. Whilst L-THE has shown promising results as a derivative to modulate immune function, it is not suitable to promote the ingestion of L-THE as a monotherapy to support the immune system in a state of infection. Appropriate medical advice and clinically tested medicine should always be encouraged as a first line management option to combat disease. Therefore, additional larger studies should be undertaken to provide conclusive clinical evidence regarding its efficacy as an individual and alternative treatment option to manage infections.

Table 3. The effect of L-THE consumption on immune function.

Impact on	Proposed Effect	Reference
Immune function	Co-administration of L-THE (280 mg) and cysteine (700 mg) attenuated an increase in neutrophil count and a reduction in lymphocyte count during exercise (in 16 athletes).	[73]
Immune function	Supplementation with L-THE (200 mg) decreases the incidence of cold and flu symptoms through enhancement of human γ and δ T lymphocyte function.	[74]
Immune function	Co-administration of L-THE (280 mg) and cysteine (700 mg) before vaccination enhanced immune responses to influenza vaccine in elderly subjects with low serum total protein or haemoglobin.	[75]
Immune function	Co-treatment of L-THE (280 mg) and cysteine (700 mg) for 2 weeks restored the attenuation of natural killer cell activity in well trained men	[77]
Immune function	Co-administration of L-THE (70 mg) and cysteine (175 mg) in 176 subjects correlated with a lower incidence in development of the common cold	[72]
Preventative immune function	Co-administration of L-THE (280 mg) and cysteine (700 mg) reduced neutrophil counts, maintained high-sensitivity CRP (hs-CRP) levels and prevented a decrease in lymphocytes post endurance training compared with placebo.	[78]
Post-operative recovery	Co-administration of L-THE (280 mg) and cysteine (700 mg) during a randomised, single blind, parallel-group trial alleviated post-gastrectomy inflammation in patients that have undertaken distal gastrectomy for cancer.	[76]

10. Cancer

The incidence of cancer in Australia and many other parts of the world is a major public health concern. In the United States alone, one in four deaths are cancer related with the highest numbers being from prostate and breast cancers for men and women, respectively [79]. Evidence from the literature proposes a potential effect of L-THE across a range of cancer cell line models, which could potentially form part of the development of newer therapeutic applications (Table 4). Currently, there have been more than 2000 papers that report the effects of tea and/or tea components against cancer [80]. However, there is currently limited clinical evidence supporting the use of pure L-THE in cancer suppression or treatment.

Table 4. The effect of L-THE on cancer cells.

Study Type	Proposed Effect	Reference
Ex Vivo*/*In Vitro		
Cancer suppression	Administration of L-THE (400 μg/mL) was found to induce cell death of four cancer cell lines: breast 23 (MCF-7), colon (HT-29), hepatoma (HepG2), and prostate (PC-3) as well as normal human liver cells *in vitro* or *ex vivo*.	[81]
Tumour growth	Dendritic cells were purified with L-THE solution (200 μmol/L) that resulted in partial recovery of dendritic cell function, promoted the differentiation of T cells and activation of cytotoxic T lymphocytes.	[82]
Cancer suppression	Four theanine derivatives (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) significantly inhibited lung cancer cell migration, growth of lung cancer and leukemia in *in vitro*, *ex vivo* and *in vivo* models of human and mouse cancers.	[83]
Cancer suppression	48-h L-THE treatment induced *in vitro* and *ex vivo* growth of human non-small cell lung cancer A549 and leukemia K562 cell lines in dose- and time-dependant manners	[80]

L-THE has shown positive effects against low sensitive tumours in combination with the chemotherapy drug doxorubicin (DOX) and has been shown to increase DOX induced efficacy through an increase in the DOX concentrations in metastatic cells upon oral dosage and intraperitoneal injection in murine models [84–86]. However, further investigation, especially in human trials, is needed to provide sufficient clinical evidence regarding the importance of these drug-tea interactions and to validate the use of L-THE as a potential adjunctive therapy in medical practice.

11. Vascular System

L-THE intake has numerous localised effects such as causing increases in body surface temperature due to relaxation of the vasculature, effects that are reliant on catecholaminergic and serotonergic neurons in both the brain and the peripheral nervous systems [5,87]. A recent double-blind, placebo-controlled trial revealed this effect was also seen when L-THE was combined with an amount of caffeine equivalent to one to two cups of tea; the L-THE was found to counteract the vasoconstrictive effects that caffeine produces [88]. Similarly, two further studies also achieved attenuation in blood pressure increases with a dosage of 200 mg L-THE: in one study in response to a high stressor in adults [89] and in the other, L-THE halted the effect of 250 mg caffeine and resulted in a decrease in blood pressure [90]. L-THE has also been shown to increase the vasodilatory capabilitiy in arteries via an increase in nitric oxide production in *ex vivo* models [91]. Consequently, L-THE consumption may result in a relaxed state associated with a decrease in blood pressure, which may be due to L-THE's vasodilation of blood vessels via the peripheral nervous system [5].

12. Applications in Food

The potential application of L-THE in functional foods, such as in teas or imbedded in another food matrix, is mainly due to its beneficial effects of potentially increasing cognitive function and relaxation [8]. Often, foods are no longer consumed to only satisfy hunger and provide the necessary nutrients for survival, but are used as a prevention strategy against nutrition related diseases and to increase overall consumer wellbeing [92]. The term "functional food" was first used in the mid-1980s in Japanese culture to describe food products fortified with beneficial constituents having the capability to deliver advantageous physiological outcomes beyond their nutrient content. They can beneficially affect one or more functions in the body, beyond the capabilities of the existing food nutrients, and can improve the state of health and well-being and/or demonstrate a reduction in disease [93]. The importance of the topic is highlighted in the recent literature, especially with respect to mandatory folate fortification in staple foods for pregnancy in women to help avoid the development of neural tube defects [94,95].

In regards to current products containing L-THE, one particular company manufactures a chewing gum called Neurogum™ which contains L-THE (60 mg/serve). This product has been promoted with claims linked to reducing anxiety, blood pressure benefits and augmenting the clinical effects of cancer drug therapy [96]. More recently, L-THE (200 mg) was administered in the form of a beverage to 36 healthy participants to test for potential beneficial stress-related effects [97]. This study found a decrease in stress responses to a multi-tasking cognitive stressor and a reduction in the cortisol response, which further supports the anti-stress effects of L-THE [97]. Another study looked into the effects on blood pressure of chocolate containing L-THE (50–200 mg per serve of chocolate) [98]. This study revealed the inhibitory sympathomimetic effects of cacao (60% w/w) and found that blood pressure was acutely lowered with the addition of L-THE into the food product.

Recently, the antiglyco-oxidative properties of L-THE obtained from decaffeinating tea powder were shown in a study looking at the formation of Maillard reaction products and the sensory attributes of breads [99]. This European study suggested a potential new dietary application in bread for L-THE derived as a by-product during the decaffeination process of tea. Furthermore, it was revealed that the product contained the same quantity of bioactives as the decaffeinated tea in terms of antioxidant polyphenols and the amino acid L-THE.

The potential use of L-THE to treat tobacco addiction as a component of cigarette filters has also been investigated [100]. Participants using tobacco-filters containing tea displayed a 56.5% decrease in cigarette use and 31.7% of the subjects ceased smoking. This highlighted the potential of using L-THE to treat tobacco related addiction and suggested a potential blocking of the nicotine receptors in the brain [100]. According to the US FDA, other potential uses include L-THE is as a food ingredient in juices, non-herbal teas, sporting beverages, specialty bottled waters, chewing gum, mints and chocolate bars [101].

As L-THE has the ability to induce a relaxed state, there is a potential market for its usefulness as an ergogenic aid in the field of high performance sports, perhaps as a co-ingredient in a sport supplement (e.g., with slow release proteins such as casein which effectively stimulate muscle protein synthesis to enhance recovery practices) [102]. Similarly, there is a gap in the current literature in respect to whether L-THE could have synergistic effects with sleep enhancement medications, such as Estazolam or Eszopicl, that are used to treat insomnia. The use of L-THE may augment the effects on these current drug therapies for sleeping disorders and lead to a reduction of the required doses and hence, may lower the potential for side effects due to the medications.

13. Conclusions

L-THE is a predominant amino acid found in tea leaves that contributes to the taste sensation called "*umami*". The methods of L-THE synthesis (synthetic and biosynthetic) vary in their yield and commercial viability; most are labour intensive and time consuming and have relatively low yields of L-THE. Apparently, no current technique offers an environmentally sustainable and economically viable method for commercial production of purified L-THE. As a result, there is a considerable demand for further research in devising newer extraction and purification procedures to reduce the environmental impact and manufacturing cost of producing L-THE [8]. In addition, there is immense interest in L-THE as a supplement as well as a functional food ingredient for its therapeutic health benefits, including but not limited to its effects on learning ability, immune function, cancer suppression and vascular relaxation. L-THE also promotes the generation of α-waves in the brain, inducing a state of relaxation without causing a state of drowsiness. Therefore, further research is warranted to develop L-theanine as a functional food additive and to explore its role in disease prevention and health promotion.

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Abbreviations

The following abbreviations are used in this manuscript:

L-THE	L-Theanine
MIP	Molecularly imprinted polymer
HPLC	High performance liquid chromatography
DAD	Diode array detectors
ECC	Electrokinetic capillary chromatography
FDA	Food and Drug Administration
AD	Alzheimer's disease
Ig	Immunoglobulin
IL	Interleukin
DOX	doxorubicin
GSH	glutathione
CV	Cardiovascular

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