Review

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L-Theanine: properties, synthesis and isolation from tea

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Abstract

Theanine is a non-protein amino acid that occurs naturally in the tea plant (*Camellia sinensis*) and contributes to the favourable taste of tea. It is also associated with effects such as the enhancement of relaxation and the improvement of concentration and learning ability. It is also linked with health benefits including the prevention of certain cancers and cardiovascular disease, the promotion of weight loss and enhanced performance of the immune system. Thus, there has been a significant rise in the demand for theanine. While theanine has been chemically and biologically synthesised, techniques to isolate theanine from natural sources remain an important area of research. In this review article, the properties and health benefits of theanine are summarised and the synthesis and isolation of theanine are reviewed and discussed. Future perspectives for the isolation of theanine from natural sources are also outlined.

Keywords: health benefits; isolation; properties; synthesis; tea; theanine

INTRODUCTION

Theanine is an abundant non-protein derived amino acid that was first isolated from green tea leaves in the late 1940s by Sakato.[1] Theanine has been named as 2-amino-4-(ethylcarbamoyl) butyric acid by the International Union of Pure and Applied Chemistry (IUPAC). However, it has also been referred to as γ-glutamylethylamide, 5-*N*-ethylglutamine, γ-glutamyl-L-ethylamide, γ-ethylamino-L-glutamic acid and γ-L-glu-ethylamide.[2–5] Similar to other amino acids in nature, theanine is a chiral species and occurs in nature predominantly as the L-(*S*) enantiomer (Fig. 1), while synthetic theanine is normally prepared as a racemic mixture of L- and D-forms.[2]

Theanine is considered to be a unique amino acid in nature because, with the exception of being found in the basidiomycete mushroom *Xerocomus badius*, its occurrence appears to be limited to the *Camellia* genus, mostly the tea-producing plants *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica* and some closely related species such as *C. japonica* and *C. sasanqua*.[6] In the leaves of the tea plant species, theanine accounts for about 500 g kg^{-1} of the free amino acids. Many of these amino acids are involved in producing the distinctive aroma and taste of tea and theanine has been linked with giving tea its distinctive umami taste.[7] Because of its contribution to taste, the theanine content in tea leaves correlates highly with tea quality and price; the teas with a high content of theanine are normally evaluated as having a higher quality and thus command a higher price.[8]

Theanine occurs in the cotyledons, shoots and roots of the tea plant seedling and it is biosynthesised from glutamic acid and ethylamine via the enzyme theanine synthetase (Fig. 2).[4] In mature plants, biosynthesis occurs mostly in the roots, from where it is transferred, via the phloem, through the stem to the growing shoots where it subsequently accumulates in the developing leaves. In the leaves, theanine can be hydrolysed back to its parent constituents through exposure to sunlight and heat. The ethylamine liberated as a result of this reaction is then utilised as a precursor in the synthesis of catechins.[9] Consequently, tea growing in climatic conditions of reduced sunlight has been shown to develop higher concentrations of theanine and lower amounts of catechins.[8] Trials to boost theanine levels in tea through controlled exposure to sunlight (e.g. with the use of shade cloth) have also been successful.[6]

Theanine constitutes between 10 and 30 g kg^{-1} of the weight of dry tea leaf.[2] However, the level of theanine varies in accordance with a variety of factors, including growing location and method of cultivation, tea grade and variety and time of harvest. As mentioned previously, tea growing in shaded areas or in areas of reduced exposure to direct sunlight generally contains higher levels of theanine.[10] Tea variety is also important, with the *C. sinensis* var. *sinensis* known to contain higher concentrations of theanine compared to *C. sinensis* var. *assamica*.[8] Also, tea harvested in early summer possesses a higher theanine content compared with tea harvested in late summer.[8] The latter may explain why theanine levels differ significantly between grades of tea from the same species and growing location. For example, Ceylon black tea (grade Pekoe) from Sri Lanka has a higher theanine content than Ceylon black tea (grade Broken) from the same growing region.[11] Post-harvest processing has little impact on theanine as green (or non-fermented) tea contains similar levels of theanine when compared to oolong (semi-fermented) and black (fermented) teas.[11]

Several *in vivo* and epidemiological studies have shown that theanine consumption can enhance health and well-being by influencing factors such as stress levels, improvements in learning

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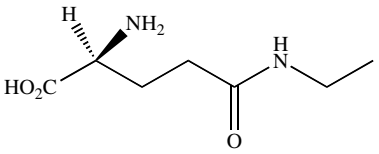

Figure 1. Chemical structure of L-theanine.

ability, prevention of cancers and vascular diseases, promotion of weight loss and enhancement of immune response.[2,6,12–30]

However, in these studies the beneficial effects were observed with daily consumption of pure L-theanine of at least 50 mg, an amount equivalent to a minimum of three cups of tea (Table 1). Furthermore, many of these studies were done using between 150 and 250 mg L-theanine, doses which would not be easily achieved even by the most avid tea drinkers; these doses would only be achieved when drinking between nine and 15 cups of tea per day. Moreover, for some people, the caffeine present in tea can cause gastrointestinal tract irritation or sleeplessness thus limiting their tea consumption and making it very difficult for them to obtain the doses of L-theanine linked with beneficial effects.[14]

Therefore, based on the doses of pure theanine used in the studies showing beneficial effects (Table 1), there is a perceived demand for L-theanine as a supplement or food ingredient.[2,6] This has spawned exploration for efficient and economical extraction techniques for the isolation of natural theanine and investigation into efficient synthetic and biosynthetic means of producing the amino acid. These approaches have become central to expanding the commercial availability of theanine for inclusion in supplements and in processed foods and beverages.

HEALTH BENEFITS OF THEANINE

After oral administration, theanine is quickly absorbed into the bloodstream through intestinal absorption, from whence it is transported to the major organs of the body, including the brain. Theanine reaches a maximum concentration in the blood between 30 min and 2 h after administration.[31] The amino acid is cleared by excretion into urine but it is also catabolised via breakdown by amide hydrolysis, yielding glutamic acid and ethylamine, with ethylamine then excreted from the body in the urine.[31]

Ingestion of theanine has been reported to facilitate the generation of alpha brain waves, which are associated with a relaxed but alert mental state.[32] In addition, theanine is reported to promote the release of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), which in turn regulates dopamine and serotonin levels in the brain.[33] Thus, theanine consumption has been closely associated with relaxation and improved learning ability (Table 1).

A recent study found that ingestion of 50 mg of L-theanine dissolved in 100 mL of water could elicit a significant effect on the general state of mental alertness or arousal in subjects by increasing alpha-wave brain activity.[15] Another study also found a link between theanine consumption (200 mg) and the reduction of anxiety.[17] Other studies have shown that consumption of theanine in combination with caffeine could further improve concentration and learning ability. For example, the intake of a combination of 250 mg L-theanine and 150 mg caffeine was found to enhance rapid simple reaction time, fast numeric working memory reaction time and improve verification accuracy during reading tasks.[19] A separate study found that consuming a combination of 100 mg L-theanine and 50 mg caffeine improved both speed and accuracy performance during attention-switching tasks performed 60 min after ingestion and reduced susceptibility to distracting information in memory tasks at 60 and 90 min following ingestion.[20]

Moreover, it is thought that theanine may provide effective prophylaxis and treatment for Alzheimer's disease as it has been reported to exert neuroprotective effects through inhibition of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors and its related pathways in a transgenic neuronal cell model.[34]

Theanine has also recently been linked to cancer prevention. Liu *et al*.[22] found that theanine was linked to the inhibition of the *in vivo* and *ex vivo* growth of human non-small cell lung cancer and leukaemia cell lines. In another study, Friedman *et al*.[23] found that theanine intake was associated with the induction of apoptosis in four cancer cell lines of breast, colon, hepatoma and prostate origin.

In addition to enhanced antitumour activity, theanine can reduce the adverse effects of the cancer treatment drug, doxorubicin by providing protection against damage caused by doxorubicin to normal tissue.[35] It also acts as a biochemical modulator to improve the therapeutic efficacy of doxorubicin by suppressing the efflux of the drug from cancer cells, thereby increasing the effective doxorubicin concentration in the tumour.[36]

Recent studies have also found that theanine was linked with regulation of blood pressure, promotion of weight loss and improvement of the immune system.[26,29] Injection of L-theanine at a dose rate of 2 g kg^{-1} was found to significantly reduce blood pressure in spontaneously hypertensive rats.[25] In humans, consumption of a single dose of 200 mg of theanine was also found to reduce blood pressure and, more importantly, theanine was found to antagonise the negative effect of caffeine increasing blood pressure, when the latter was consumed as a single 250 mg dose.[26]

In addition, co-administration of L-theanine and L-cystine was reported to enhance antigen-specific immunoglobulin G (IgG) production, partly through augmentation of glutathione (GSH) levels and T-helper cell (Th2)-mediated responses.[27] Similarly, co-treatment of L-theanine with L-cystine was found to improve the immune response via an increase in GSH production, which significantly prevented weight loss associated with infection in aged mice.[29]

In summary, findings from several studies have revealed that theanine not only contributes to a favourable flavour in tea but also provides significant health and cognitive benefits. No studies

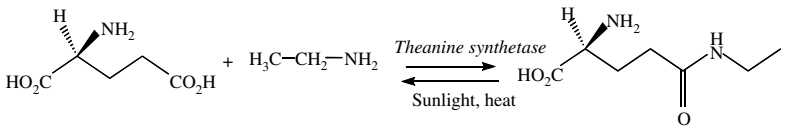

Figure 2. Biosynthesis and decomposition of L-theanine in the tea plant.

Table 1. Beneficial effects of theanine

Impact on	Report of beneficial effects	Ref.
Relaxation effects	Intake of 200 mg of L-theanine dissolved in 100 mL of water generated α-brain waves in female volunteers aged from 18 to 22 years.	6
	Ingestion of 50 mg of L-theanine produced greater and increased α-activity in young participants.	15
	Administration of 200 mg of L-theanine dissolved in 100 mL of water resulted in the generation of α-brain waves in female university students.	16
	Ingestion of 200 mg of L-theanine had some relaxing effects under resting conditions in male and female university students.	17
	L-theanine intake resulted in a reduction in heart rate and salivary immunoglobulin A responses to an acute stress task in male undergraduate students.	18
Improvement in learning ability	Intake of a combination of 150 mg of caffeine and 250 mg of L-theanine improved reaction time, working memory and sentence verification accuracy in participants aged 18–34 years.	19
	Ingestion of a combination of 100 mg of L-theanine and 50 mg of caffeine enhanced speed and accuracy of performance in the attention-switching task.	20
	Ingestion of 250 mg of L-theanine enhanced the difference between the effects of visual versus auditory stimuli on the α-wave activity over the parieto-occipital scalp.	21
Cancer prevention	Theanine was found to inhibit the *in vivo* and *ex vivo* growth of human non-small cell lung cancer A549 and leukaemia K562 cell lines.	22
	Theanine treatment at 400 $\mu g\ mL^{-1}$ was found to induce cell death of four cancer cell lines: breast (MCF-7), colon (HT-29), hepatoma (liver) (HepG2), and prostate (PC-3).	23
	Theanine (40 mg mL^{-1} at a dose of 1 mL 100 g^{-1} of rat) could inhibit invasion of the receptor-mediated cancer cell line AH 109A.	24
Prevention of vascular diseases	Theanine injection (200 g kg^{-1}) reduced blood pressure in spontaneously hypertensive rats.	25
	Theanine consumption (200 mg) antagonised the effect of caffeine (250 mg) on blood pressure in healthy adult participants.	26
Improvement of immune system	Co-administration of L-theanine (8 g kg^{-1}) and L-cystine (20 g kg^{-1}) enhanced antigen-specific IgG production partly through augmentation of GSH levels and Th2-mediated responses.	27
	Co-administration of L-cystine (700 mg day^{-1}) and L-theanine (280 mg day^{-1}) before vaccination enhanced the immune response to influenza vaccine in elderly people with low serum total protein or haemoglobin.	28
Effects on weight	Co-treatment of L-theanine and L-cystine to aged mice improved immune response and prevented the weight loss associated with infection.	29
	Theanine (30 g kg^{-1} diet) and caffeine (50 g kg^{-1} diet) were responsible for the suppressive effect of a green tea powder on body weight increases and fat accumulation in mice.	30

associated with theanine toxicity in human or animal models have been reported. However, the US Food and Drug Administration (FDA) recommends that the total daily consumption of theanine should not exceed 1200 mg.

Because of its contribution to favourable flavour and health benefits, theanine has great potential for utilisation as a food ingredient or as a dietary supplement.[2,6] Future studies should therefore further explore the utilisation of theanine as a food additive and the potential impacts of prolonged theanine consumption on human health.

PHYSICAL AND CHEMICAL PROPERTIES OF THEANINE

The major physical properties of theanine are described in Table 2. Theanine, like the protein-based amino acids, exists as a zwitterionic species and is a colourless crystalline solid (needles, melting point 214–216 °C).[2] Studies on the buffering capacity of green tea extracts suggest the pK_a of the theanine amino group to be 8.9. The pK_a of the carboxyl unit was not formally quantified due to interference from other acidic species. However, comparisons with close structural analogues such as glutamine suggest the value lies in the range 2.1–2.5.[37]

Table 2. Major physical properties of theanine[2,14]

Property	Description
Molecular formula	$C_7H_{14}N_2O_3$
Molecular weight	174.2 g mol^{-1}
Melting point	217–218 °C
Appearance	Crystallises in needle form
Colour	Colourless
Solubility	Soluble in water and insoluble in ethanol, methanol, chloroform and ether
Stability	Stable in acidic and unstable in alkaline conditions
Taste	Umami taste with little or no aftertaste

Theanine is stable under acidic conditions but undergoes base hydrolysis to yield glutamic acid and ethylamine.[2,11] During infusion, theanine does not react chemically with any of the other tea components. This is in contrast to catechins, which can precipitate from solution as a result of π stacking interactions with caffeine,[38,39] or can react with proteins and enzymes such as lipoxygenase, α-amylase, pepsin, trypsin and lipase.[38]

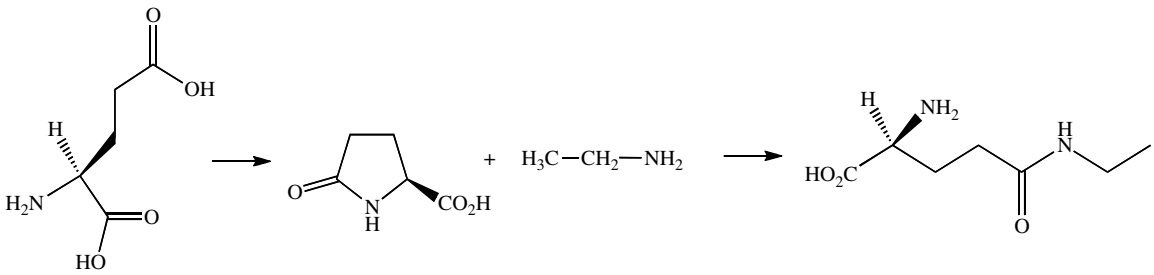

Figure 3. Chemical synthesis of L-theanine from L-glutamic acid.

Of the tea components, theanine exhibits a higher water solubility (385 g L^{-1} at 0 °C, 556 g L^{-1} at 100 °C) than caffeine (21.7 g L^{-1}) and the catechins (e.g. epigallocatechin gallate, 5 g L^{-1}); this permits a very effective diffusion of theanine from tea during hot-water infusions.[2,40] The relative insolubility of theanine in organic solvents such as methanol and chloroform (Table 2) allows for its easy separation from caffeine and the catechins, which possess a molecular rather than a zwitterionic structure.[2]

Theanine has a complex umami taste.[41–43] It also exhibits a synergism with the common umami flavouring agents monosodium glutamate and the purine nucleoside inosine 5′-monophosphate, which leads to an enhancement of the umami taste experience.[41] The term umami is a Japanese-derived expression and it is classified as the fifth taste after sweet, salt, bitter and sour.[42,43] Most of the typical umami substances are divided into two groups: L-α-amino acids, usually represented by monosodium glutamate and 5′-ribonucleotides and their derivatives, usually represented by inosine 5′-monophosphate or disodium 5′-guanylate.[42,43]

ANALYTICAL METHODS FOR THEANINE

Several analytical methods have been developed for identification and quantification of theanine, which can be employed for determining theanine concentration, production yield or theanine purity in final products. Analysis of theanine has been mainly facilitated by chromatographic techniques such as high-performance liquid chromatography (HPLC), capillary electrophoresis and micellar electrokinetic chromatography.[11,44,45] Peng *et al.*[46] developed a method to determine theanine and other tea components by HPLC using an amide-C16 column coupled with a UV detector (210 nm and 280 nm). Syu *et al.*[47] developed an improved detection method to quantify theanine by derivatising with the chromophoric labelling reagent dabsyl chloride (4-dimethylaminoazobenzene-4′-sulfonyl chloride) to form dabsyl-theanine, which was subsequently analysed by reverse phase HPLC (C18 column, 425 nm).

Chen *et al.*[44] used capillary electrophoresis conducted with an uncoated fused-silica capillary column coupled with a diode array detector (214 nm) for determination of the theanine concentration. Li *et al.*[48] used micellar electrokinetic capillary chromatography, which was conducted on an open tubular fused-silica capillary column coupled to a UV detector set at 360 nm. Hsiao *et al.*[45] also used micellar electrokinetic capillary chromatography for quantification of theanine, although with a slightly different system and analysis conditions. The system used an untreated fused-silica capillary column and a built-in photodiode array detector set at 200, 205, 220, 266 and 280 nm. In addition, theanine has also been determined using isotachophoretic anion-exchange chromatography with integrated pulsed amperometric detection or enzymatic flow injection analysis.[49–51]

CHEMICAL SYNTHESIS AND BIOSYNTHESIS OF THEANINE

Chemical synthesis of theanine

Theanine was first chemically synthesised in 1942 by Lichtenstein[52] with a yield of 90 g kg^{-1} by treating pyrrolidone-5-carboxylic acid with aqueous ethylamine for 20 days at 37 °C. A number of other synthetic approaches have since been developed including a large-scale production method involving the reaction of γ-benzyl glutamate in the presence of trityl chloride and ethylamine (339 g kg^{-1})[53] and a two-step approach involving initial dehydration of L-glutamic acid to L-pyrrolidone carboxylic acid followed by ring opening in the presence of ethylamine to yield theanine (374 g kg^{-1}) (Fig. 3).[54]

More recently, theanine was produced in four steps starting from commercially available *N*-phthaloyl-L-glutamic acid, which was dehydrated to the corresponding cyclic anhydride by reaction with acetic anhydride and then the ring was opened by reaction with ethylamine. Subsequent deprotection of the amine unit with hydrazine hydrate gave theanine with a 700 g kg^{-1} overall yield.[55]

Chemical synthesis of theanine offers a simple, convenient and cost-effective alternative to direct extraction of the amino acid from natural sources or preparation by biosynthetic methods.[56] However, limitations exist for the use of synthetic theanine as a food supplement or ingredient because of increasing consumer resistance to the inclusion of so-called 'non-natural' additives in the diet. In addition, theanine produced by some processes requires protection and de-blocking procedures for its reactive groups,[2,56] which can add considerable time and cost to the synthesis of the amino acid. However, the main limitation faced by synthetic theanine is that it is normally prepared as a racemic mixture of L- and D-forms.[2] Similar to other amino acids in nature, theanine is a chiral species and occurs in nature predominantly as the L-(*S*) enantiomer (Fig. 1).

Biosynthesis of theanine

In the tea plant, theanine is biosynthesised from glutamic acid and ethylamine by the enzyme theanine synthetase (Fig. 2).[4] However, the enzyme is very labile and cannot be used to produce the amino acid in commercial quantities.[56] Therefore, other methods for the enzymatic synthesis of theanine have been developed using bacterial enzymes such as glutaminase, glutamine synthetase and γ-glutamyltranspeptidase (Table 3, Fig. 4).

A glutaminase from *Pseudomonas nitroreducens* has been used which can simultaneously hydrolyse glutamine to ammonia

Table 3. Biosynthesis of theanine using bacterial enzymes

Enzyme	Brief description	Ref.
Glutaminase	The glutaminase isolated from *Pseudomonas nitroreducens* IFO 12 694 was incubated for 7 h at 30 °C and pH 11 to simultaneously hydrolyse glutamine to glutamic acid and to react with ethylamine to form theanine.	58
	Theanine was continuously synthesised from glutamine and ethylamine using immobilised *P. nitroreducens* IFO 12 694 cells for 12 days at 30 °C and pH 9.5.	59
Glutamine synthetase and related enzymes	Theanine was synthesised from glutamic acid and ethylamine using a glutamine synthetase from *P. taetrolens* Y-30 with sugar fermentation by baker's yeast cells as an ATP-regeneration system.	61
	Theanine was formed from glutamic acid and ethylamine using a γ-glutamylmethylamide synthetase from *Methylovorus mays* coupled to yeast sugar fermentation for ATP regeneration.	62
	Theanine was synthesised from glutamic acid and ethylamine using a glutamine synthetase from *Bacillus subtilis* coupled with an alcoholic fermentation system.	63
γ-Glutamyl-transpeptidase	Theanine was synthesised from glutamine and ethylamine using γ-glutamyltranspeptidase from *Escherichia coli* k-12 SH642 at pH 10 and 37 °C over 2 h.	64
	The γ-glutamyltranspeptidase from *E. coli* k-12 MG1655 catalysed the reaction between glutamic acid γ-methyl ester and ethylamine to form theanine at pH 10 and 45 °C over 8 h.	65

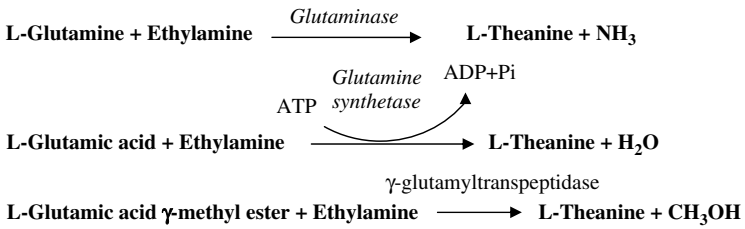

Figure 4. Biosynthesis of L-theanine using bacterial enzyme systems.[56].

and glutamic acid and catalyse the reaction of glutamine with ethylamine to form theanine.[57–59] However, the use of glutamine in preference to glutamic acid as the starting material in this process suffers from the disadvantage of being more expensive, more time consuming and from glutamine being less stable.

The enzyme glutamine synthetase and related enzymes, originating from *Escherichia coli*,[60] *Pseudomonas taetrolens*,[61] *Methylovorus mays*[62] and *Bacillus subtilis*,[63] have been utilised for the synthesis of theanine from glutamic acid and ethylamine. Unlike the glutaminase biosynthetic pathway discussed previously, this glutamic acid transformation, while using a cheaper and more stable starting material, requires a continuous supply of ATP to drive the reaction.[62] Another non-ATP-dependent biosynthetic pathway, involving γ-glutamyltranspeptidase from *E. coli* and glutamine as the starting substrate, has also been successfully developed but the reaction requires a high concentration of ethylamine to drive the conversion of glutamine to theanine.[56,64,65]

Despite some limitations, theanine biosynthesis shows great potential as an industrial-scale preparation method. Enzymes are also stereospecific and offer the advantage of producing the naturally occurring L-(*S*) enantiomer of theanine (Fig. 1) thereby overcoming a drawback that synthetic production techniques have in producing a racemic mixture of L- and D-forms.[2] However, future studies need to focus on method optimisation parameters including product purity, yield maximisation and the reduction of production costs associated with scale-up procedures.

Table 4. Composition of soluble components in dry tea leaf[7]

Component	Concentration (g kg^{-1})
Polyphenols	360
Caffeine	30
Theanine	15–20
Protein	150
Chlorophyll	5
Organic acids	15

ISOLATION OF NATURAL THEANINE FROM TEA

Extraction methods for theanine from tea

There has been an increase in demand for natural foodstuffs and food additives perceived to impart therapeutic benefits. Similarly, the stress-relieving and other beneficial properties reported for theanine (Table 1) has led to a rising demand for naturally sourced extracts of the amino acid.[2,64] Therefore, significant research has been directed towards optimising theanine extraction from tea leaves. However, isolation of theanine in high purity presents a considerable challenge due to the presence of other soluble substances, such as caffeine and polyphenols, in significant concentrations (Table 4).

Several attempts have been made to isolate theanine from tea but these methods still have limitations. For example, Zhang *et al*.[66] isolated theanine from green tea by using preparative HPLC. The catechins were initially extracted from green tea with ethyl acetate. The theanine was then extracted from the tea with water

at 50 °C and purified using a 732 cation exchange column to yield an extract containing 500 g kg^{-1} theanine. Final purification was achieved using a preparative C18 reversed-phase HPLC column with a formic acid mobile phase buffered to pH 3.0. While the final theanine purity from the procedure was high (980 g kg^{-1}), the low overall yield (2.53g day^{-1}) coupled with high production costs and limited scale up potential make this method unappealing as an industrial purification method.[66]

Lachová *et al*.[67] used molecularly imprinted polymer (MIP) technology to selectively isolate theanine from green tea extracts. Two separate MIP formulations were prepared from Nylon-6 dissolved in formic acid using phase inversion techniques. The polymers were then washed with acetic acid solution to remove the template from the generated imprint cavities. The selectivity of theanine rebinding to the MIP was then evaluated by solid phase extraction. While the theanine recovery proved satisfactory (880 g kg^{-1}), the analyte purity was not stated and appeared to be low.[67]

A number of patents have been developed covering theanine extraction. Ekamayake and Li[68] successfully developed a theanine extraction method which avoids the use of organic solvents. A hot-water extract of tea leaves was passed through a preparative column packed with a polyamide solid phase. Collection of theanine rich fractions was then undertaken. While cost-effective, the procedure yielded theanine contaminated with impurities such as saccharides, polyphenols and caffeine. Another patent by Baudouin[69] reported purifying theanine by extracting black tea with water, then filtering and drying the extract to obtain a theanine-rich solid. The extract was then redissolved and purified by fractionation using cation exchange chromatography with Diaion UBK550. However, the final theanine purity was relatively low (440 g kg^{-1}) and the production procedure was complicated and long.

In summary, several attempts have been made to isolate natural theanine from tea. While variable in success, all methods involve laborious and time consuming protocols that give relatively low yields of theanine. None of the methods outlined offer a reliable and cost-effective procedure for commercial-scale harvesting of high purity theanine. Therefore, there is a need for further investigation of extraction and purification methods for the isolation of theanine from tea.

Future perspectives for isolating theanine from tea

The starting material for the isolation of natural theanine is abundant because world tea production is high and increasing at an annual growth rate of 2%.[70] Like other bioactive compounds in plants, theanine can potentially be extracted and isolated using several steps, which fall into several basic categories: starting material treatments, extraction of tea constituents from tea leaves into solvents, separation of theanine from other soluble components and drying of the theanine to obtain a powder (Fig. 5).

As mentioned previously, the theanine content in tea varies in accordance with the growing location, the method of cultivation, the tea grade, the tea variety and the time of harvest.[8,10,11] In order to obtain a high yield of theanine, teas possessing a high natural theanine content should be selected as the starting material for extraction and isolation. To accelerate theanine release and thereby improve extraction efficiency, dried tea should be finely ground.[71] However, it should be noted that the extraction of constituents from tea ground to small particle sizes is only quicker, compared to large particle sizes, when the solution is well agitated.[72]

Before separating it from the other components in tea, the theanine and other components are extracted into solvents. The extraction process can be efficiently conducted by employing novel extraction techniques such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), ultrahigh pressure extraction (UHPE) and subcritical water extraction (SWE).[72] In comparison with conventional extraction techniques, these novel extraction techniques possess advantages such as shorter extraction times, reduced solvent consumption, higher extraction yields and better quality final extracts.[72]

However, the conditions for each extraction technique need to be further studied to optimise the extraction of theanine. For example, MAE is a promising method for the extraction of tea constituents but the conditions for MAE, such as solvent character, volume of solvent, pre-leaching time, MAE time and microwave power, are important and have to be optimised to achieve a high extraction efficiency.[73] Similarly, SFE is also an effective extraction method for plant bioactives. However, temperature, pressure and solvent character must be carefully controlled to optimise extraction efficiencies without affecting the recovery rate.[38]

Once in solution, theanine can potentially be separated from the other tea components, such as caffeine and catechins, by employing column chromatography, membrane separation, resin adsorbency and solid phase extraction techniques. Column chromatography, such as preparative reverse-phase HPLC and high-speed counter current chromatography, has been widely used for the separation of natural products.[74,75] However, safe solvent systems for extracting bioactive compounds and for use as mobile phases in chromatographic separation procedures need to be developed to replace undesirable solvents such as chloroform, ethyl acetate and acetone, which are unsafe because of their known impacts on human health.

Membrane technology can potentially be used to separate theanine from other compounds based on their molecular size. The disadvantage of this approach is that the membranes can often foul during operation, resulting in low permeate flux rates.[76] Synthetic resin adsorbents such as macroporous polymerics and polyamides have been successfully used to isolate caffeine and catechins in tea.[77,78] The potential therefore exists for these materials to be utilised for the isolation of theanine. Alternatively, solid phase extraction (SPE) has been widely used for the separation and isolation of analytes from many different solutions, and may therefore find potential application in theanine isolation.[79] However, the materials and methodology used in SPE need to be further studied in order to improve their effectiveness and capacity for the isolation of significant quantities of theanine.

Once isolated in solution, theanine powder preparations can then be obtained through drying processes; theanine is stable in solid form and can be transported and stored for long periods without loss of quality.[80] However, further studies are required to identify the optimal drying processes and conditions to minimise energy consumption and production costs.

CONCLUSIONS

Theanine is a unique amino acid predominately found in tea where it contributes significantly to the taste quality of the infusion. Theanine has also been associated with benefits on human function

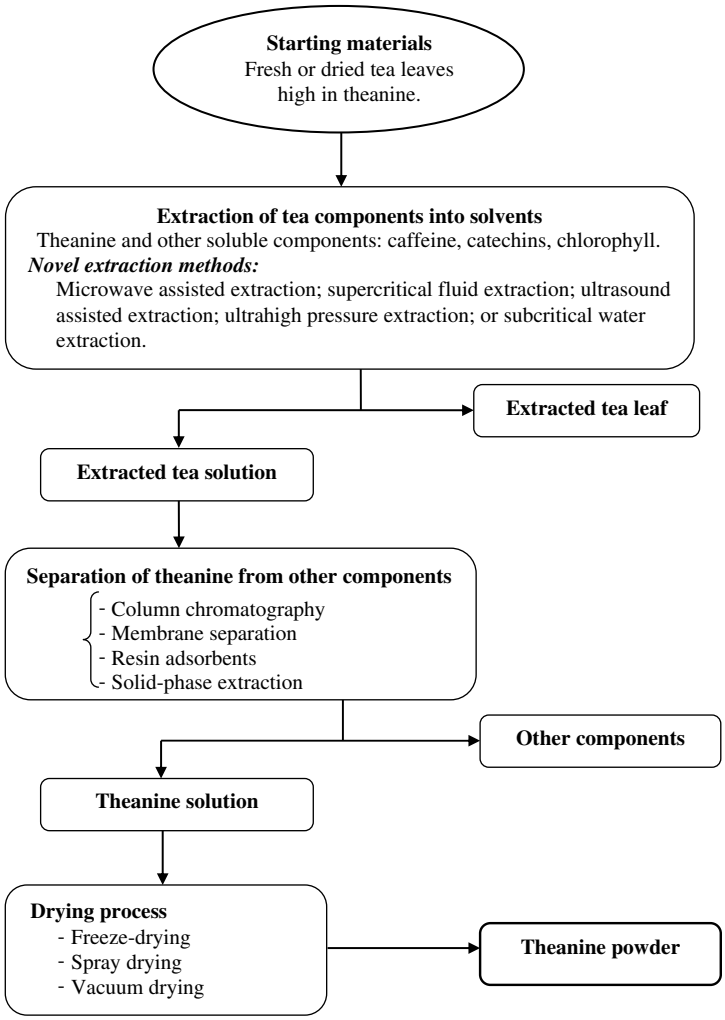

Figure 5. Diagram for the prospective extraction and isolation of theanine from tea.

and health such as enhancement of relaxation, improvement of learning ability, prevention of cancer and cardiovascular disease, promotion of weight loss and improvement of the immune system. Due to its favourable contribution to taste and health benefits, there is a perceived increasing demand for its use in dietary supplements, food additives and functional foods in recent years. Many efforts have been made to synthesise and produce theanine to meet this demand. Synthetic and biosynthetic methods for theanine preparation have been reported, with procedures varying in complexity, yield and product purity.

Interest in more-natural health additives has also seen an increased demand for naturally sourced theanine. Isolation of theanine from tea has been accomplished. However, the methods used are generally complex, time consuming and costly. Therefore, further research is needed to develop safe, sustainable, environmentally friendly and cost-effective methods for the isolation of theanine from tea in high yield and purity.

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