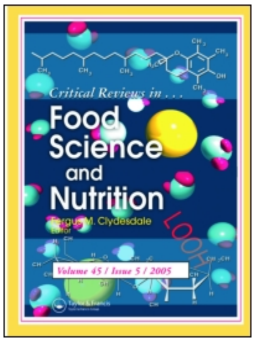

Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

L-theanine, unique amino acid of tea, and its metabolism, health effects, and safety

Duygu Türközü & Nevin Şanlier

To cite this article: Duygu Türközü & Nevin Şanlier (2017) L-theanine, unique amino acid of tea, and its metabolism, health effects, and safety, Critical Reviews in Food Science and Nutrition, 57:8, 1681-1687, DOI: 10.1080/10408398.2015.1016141

To link to this article: https://doi.org/10.1080/10408398.2015.1016141

Accepted author version posted online: 20 Jul 2015.
Published online: 20 Jul 2015.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
2017, VOL. 57, NO. 8, 1681–1687
http://dx.doi.org/10.1080/10408398.2015.1016141

Taylor & Francis
Taylor & Francis Group

L-theanine, unique amino acid of tea, and its metabolism, health effects, and safety

Duygu Türközü and Nevin Şanlier

Gazi University, Faculty of Health Sciences, Nutrition and Dietetics Department, Ankara, Turkey

ABSTRACT
Tea has been a very popular beverage around the world for centuries. The reason that it is delicious, enabling hydration, showing warming and relaxing effect can be mentioned why it is consumed so much in addition to its prominent health effects. Although the catechins and caffeine are the primary bioactive components that are related with the health effects of the tea, the health effects of theanine amino acid, which is a nonproteinic amino acid special to tea, has become prominent in recent years. It has been known that the theanine amino acid in tea has positive effects especially on relaxing, cognitive performance, emotional status, sleep quality, cancer, cardiovascular diseases, obesity, and common cold. The results of acute and chronic toxicity tests conducted on the safety of theanine express that L-theanine is reliable in general even if it is consumed too much with diet. However, it has not revealed a clear evidence-based result yet regarding theanine metabolism, health effects, and its safety. Within this frame, chemical structure of theanine, its biosynthesis, dietary sources, metabolism, health effects, and safety are discussed in present study.

KEYWORDS
Tea; theanine; health; safety

Introduction

Hot infusion of the tea plant, which is a member of Theaceae family, has been a worldwide popular beverage for centuries in terms of both of its production and consumption. According to the recent World Tea Market 2013 Report, tea consumption in the world has been second in rank after water consumption (Anonymous, 2013). Tea is widespread in Turkey as well and it was established with Turkey Nutrition and Health Survey (2010), 92.3% of adult individuals in the urban areas consume tea daily where the percentage was 94.5% in rural areas (The Republic of Turkey Ministry of Health and Hacettepe University, 2010). In addition to reasons such as its popular consumption, being tasty, enabling hydration, and showing warming and relaxing effects, tea has been consumed significantly more because its health effects have come forward especially in the recent years. With the recent in vivo, in vitro, animal, and human studies, it was stated that tea had significant health effects in noncontagious chronic illnesses such as cardiovascular, cerebrovascular illnesses, diabetics, some cancer types, obesity, etc. (Lin et al., 2003; Vuong, 2014).

Today, various types of tea are produced by using different processing techniques. Black tea, green tea, white tea, oolong, and pu-erh are the main five types of tea which are produced in common (da Silva Pinto, 2013). Black tea is produced by getting it dried after proper oxidation of ripe tea leaves during withering, curling, and fermentation processes. Green tea is produced by converting all the enzymes in an inactive form through short term heat shock without applying withering and fermentation processes on the ripe tea leaves (Kobayashi and Ikeda, 2014). White tea is a type of tea which is produced by withering and drying the leaves or buds of unripe tea plant and whose oxidation is kept at a minimum level (Hilal and Engelhardt, 2007). Oolong tea is produced through withering, partial fermentation, and drying of ripe tea leaves while pu-erh, or pu'er with its other name, is produced like black tea; however, fermentation time is longer to the unripe tea leaves during its production (Jain et al., 2013).

Among the types of tea, especially black tea and green tea are the two types of tea studied most in terms of their bioactive components (da Silva Pinto, 2013). The main bioactive components of black and green tea leaves are the flavonols and flavonol-3-ols (catechins) of flavanoids, which compose of around 35% of the dry weight of the tea. In addition, flavonol-3-ol derivative theaflavins and thearubigins, phenolic acids such as gallic acid, alkaloids such as methylxanthines (caffeine, etc.), and polyamines such as spermidines and spermines together with nonproteinic amino acids such as gamma-aminobutyric acid (GABA) and L-theanine are the main bioactive components in tea (da Silva Pinto, 2013; Jain et al., 2013). Lots of factors such as the type of the tea plant, geographic area in which it is produced, and climate conditions may affect the amounts of those bioactive components (Lin et al., 2003).

For a long time, especially catechins and caffeine come forward as the main bioactive components that are related with the health effects of the tea. However, health effects of theanine amino acid have begun to come to forefront in recent years. Theanine amino acid, which is a nonprotein in the tea, has been proved to have effects especially on relaxing, cognitive performance, emotional state, sleep quality, cancer, cardiovascular diseases, obesity, and common cold (Vuong et al., 2011).

CONTACT Nevin Şanlier, Professor nevintekgul@gmail.com Gazi University, Faculty of Health Sciences, Nutrition and Dietetics Department, Emniyet Mahallesi, Muammer Yaşar Bostancı Caddesi, No:16, 06500 Beşevler/Ankara, Turkey.

However, there is not a clear result yet on the metabolism of theanine, its health effects, and its safety as the result of long-term exposure in literature.

Theanine and its chemical structure

Theanine is a nonprotein derivative amino acid, which was isolated from the leaves of green tea by Sakato through the end of 1940s for the first time and which has a weight of 174.20 Da (Sakato, 1949). Theanine ($C_7H_{14}N_2O_3$) has been named as 2-amino-4 (ethylcarbomoil) butyric acid by International Union of Pure and Applied Chemistry (IUPAC). Theanine is also named as γ-glutamilethylamid, 5-*N*-ethylglutamin, γ-glutamic-L-ethylamide, γ-ethylamino-L-glutamic acid, and γ-L-glu-ethylamide. Similar to the other amino acids, theanine also has chiral centers. It mostly exits as L-(S) enantiomers in the nature (Juneja et al., 1999; Vuong et al., 2011).

Theanine biosynthesis

Theanine biosynthesis is made in the cotyledons, shoots, and roots of tea plant vines by glutamic acid and ethylamine theanine synthetase enzyme. And the biosynthesis in the mature tea plant mostly takes place in the roots rather than the cotyledons. This is thought to happen because the theanine synthetase gene (TS_1 and TS_2) transcriptions are rather less in the cotyledons compared to the other parts of the plant (Deng et al., 2008; Vuong et al., 2011).

The synthesized theanine is transferred to the growing plant vines by phloem and mostly stored in the growing leaves. It is hydrolyzed again in the leaves after exposure to the sunlight and temperature. Ethylamine, which occurs as the result of this reaction, is used as a precursor in catechin synthesis. As a result, the level of concentrations of theanine is high where the level of concentration of catechin is low in the tea that is grown in the climate conditions with low levels of sunlight (Vuong et al., 2011).

Sources of L-theanine

Natural sources

In the nature, L-theanine exits in *Xerocomus badius* mushroom, a kind of nonedible mushroom, partly in *Camellia genus* and mostly in *Camellia sinensis*. var. *Sinensis, C. sinensis*. var. *Assamica* is a kind of amino acid exists in tea leaves such as *C. japonica* and C. *sasanqua* (Deng et al., 2008). L-theanine contributes to the aroma of tea in a high level and especially it is related with the umami taste of the tea (Narukawa et al., 2014). L-theanine composes almost 50% of the free amino acids in the tea. The amount of L-theanine in the tea composes 1–3% of the dry tea and that amount changes according to a lot of factors such as the geographic area in which the tea is produced, production techniques, tea class, type and time of harvest, etc. (Vuong et al., 2011). Type of the tea is important as well in terms of L-theanine concentration. In general, when compared to *C. sinensis* var. *Sinensis, C. sinensis* var. *Assamica, C. sinensis* var. *Sinensis, C. sinensis* var. *Sinensis* have higher levels of L-theanine (Chu, 1997). In addition, the tea harvested in the early summer has more L-theanine compared to tea harvested in the later period of summer (Vuong et al., 2011). This fact puts light on the difference in the levels of L-theanine concentration in the same type of tea produced in the same regions.

The effect of process stages after the harvest on the L-theanine concentration is yet contradictory. In some studies, it was found out that white tea, which is not being subjected to fermentation and oxidation processes, has higher levels of L-theanine compared to the other types of tea (Alcázar et al., 2007; Zhao et al., 2011). In some studies, it was found out that green tea, which is not being subjected to fermentation process, had similar levels of L-theanine with half fermented oolong tea and fermented black tea (Ying et al., 2005; Alcázar et al., 2007). In another study, it was found out that, green tea had lower levels of L-theanine when compared to oolong tea and black tea (Ekborg-Ott et al., 1997). In another study, it was found out that 200 mL of black tea had higher levels of L-theanine (respectively: 24.2 ± 5.7, 7.9 ± 3.8 mg) compared to green tea (Keenan et al., 2011). In another study the brewing time of tea is the most important determining aspect of tea in terms of L-theanine concentration and the milk and sugar added in small quantities do not cause important differences in the concentration (Keenan et al., 2011). In another study, it was found out that a standard mug of 200 mL had 10–20 mg/L (Hilal and Engelhardt, 2007). L-theanine where in another review there was 25–60 mg/L of L-theanine in 200 mL tea types (Bryan, 2008). However, lack of having a standard for brewing tea (brewing time, portion amount and the amount of tea, etc.) is produced as the reason for not having a standard L-theanine level (Bryan, 2008).

Synthetic L-theanine (Suntheanine)

Synthetically, L-theanine (Suntheanine) is produced as racemic mixture of D- and L- forms from food borne L-glutamine and ethylamine by using glutaminase enzyme (Juneja et al., 1999). Glutaminase is mostly derived from *Pseudomonas nitroreducens* or *Bacillus amyloliquefaciens* that are nontoxic or nonpathogenic for human (European Food Safety Authority (EFSA), 2011).

L-theanine metabolism

L-theanine is a water soluble molecule with 174.20 Da weight and it is rapidly absorbed in intestinal after it is taken orally. L-theanine is transported through cotransport with Na^+ from brush border of intestine (Unno et al., 1999). In addition, it is also reported that L-theanine is carried through methionine carrier transport system through intestine. The absorbed L-theanine is transported by blood to the major organs of the body, mainly to the brain. Then, it can either be discharged directly by urine or catabolized to glutamic acid and ethylamine through amid hydrolysis in the kidneys, then it is also discharged from the body with urine (Vuong et al., 2011).

Having information on the pharmacokinetics of the L-theanine will bring light on the further studies regarding its health effects. The lag time of 25–100 mg doses taken from L-theanine as rich tea and from biosynthetic L-theanine as aqueous solution was reported as 10 minutes and their half-lives of elimination were reported as 15 and 65 minutes, respectively.

L-theanine reaches its maximum concentration in blood from 30 minutes to 2 hours after it is taken. In a study conducted by van der Pijl et al. (2010), it was reported that L-theanine reached to maximum plasma concentration (1.0–4.4 mg/L) after 50 minutes (van der Pijl et al., 2010). It was stated that serum concentration of L-theanine began to slowly drop within 24 hours (Juneja et al., 1999).

There is limited amount of studies regarding the bioefficacy of enantiomers of L-theanine (D-, L-theanine) in literature. Desai et al. (2005) evaluated the pharmokinetics of L-theanine, L-theanine and D-, L-theanine (racemic mixture) in rat plasma and urine samples and reported in the result that absorption of D-, L-theanine was less than L-theanine after it had been orally taken (Desai et al., 2005). However, there is no study reporting the effects of those enantiomers on human in the tea based formulations yet (da Silva Pinto, 2013).

Effects of L-theanine on health

When the health effects of tea are mentioned, mostly the two bioactive components, catechin and caffeine, are considered. However, recently it has been stated that, like caffeine and catechin, L-theanine amino acid is responsible of an important part of health effects of tea as well (da Silva Pinto, 2013). The studies conducted in that direction state that L-theanine amino acid is related with the physiologic and illness situations shown in Fig. 1 (Yokozawa et al., 1995; Song et al., 2003; Kelly et al., 2008; Yamada et al., 2008; Liu et al., 2009; Lyon et al., 2011; Matsumoto et al., 2011).

L-theanine and stress

Since antique ages, tea has been thought to have a relaxing effect. It has been found out that L-theanine is also responsible for the relaxing effect of tea. Normally, there are α, β, δ, and θ waves, which determine the mental state, on the surface of the brain. α wave of the brain is especially thought to be the indicator of relaxation (Juneja et al., 1999). It is stated that, 40 minutes after orally taking L-theanine (50–200 mg) α waves occur on the occipital and parietal areas of the brain and that causes relaxation without causing a state of sleep (Kobayashi et al., 1998). Song et al. (2003) found out that 200 mg L-theanine increased the alpha (α) activity in the frontal and occipital areas 40 minutes after it was taken by individuals with high anxiety levels (Song et al., 2003). However, the measurements made by Gomez-Ramirez et al. (2007) for the performances require high level of attention, it was reported that 250 mg L-theanine caused decreases in alpha (α) activity of the brain. As far as known, that study is the only one that has found decrease in alpha (α) activity related with the decrease in the reaction in the works that require audio/sensory attention among the studies examining the relation between L-theanine and the cognitive performance of humans (Gomez-Ramirez et al., 2007).

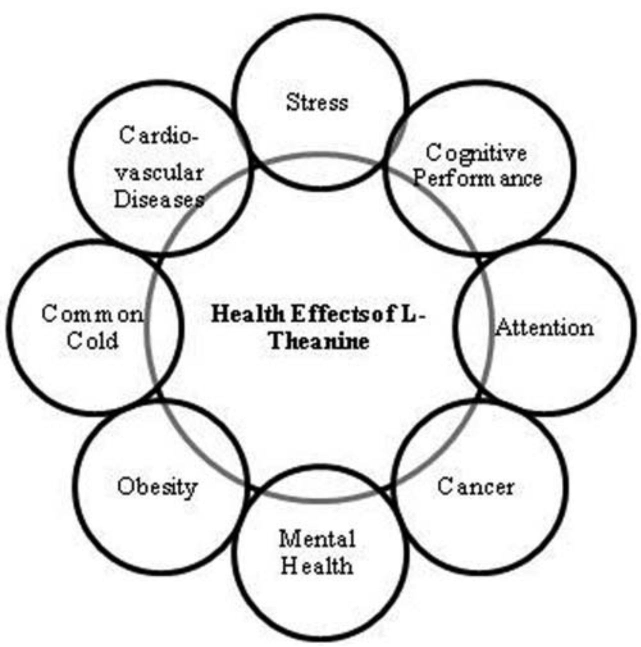

Figure 1. Health effects of L-theanine.

It is reported in some studies that L-theanine decreased the body pressure and the acute stress state. In a study supporting this result, Kimura et al. (2007) found out that 200 mg L-theanine caused decrease in heart rate and saliva IgA response, which occur as the response to acute stress. In addition, when compared to placebo, it was found out that the anxiety state and subjective perception stress level decreased in L-theanine group. Similarly, instead of 50 mg, 200 mg L-theanine caused increase in the alpha (α) waves of the brain among the participants who were relaxing (Kimura et al., 2007). In another study conducted, 14 participants (22.8 $\pm$ 2.1 years) were given L-theanine (200 mg), caffeine (100 mg) or placebo and at the end of the study it was found out that L-theanine tended to decrease the increase of blood pressure in an important amount where caffeine tended to decrease in less amount. Besides, when compared to placebo, it was found out that L-theanine caused decreases in the anxiety scores (Yoto et al., 2012). Lu et al. (2004) determined through Bond - Lader Visual Analogue Scales that L-theanine increased the level of tranquility. However, it was stated that those results are mostly obtained from rested participants and the same was not valid for the participants with increased anxiety state (Lu et al., 2004).

L-theanine and cognitive performance

L-theanine and its positive effects on cognitive performance is one of its most important functions. Because of chemical structure of L-theanine similar to glutamate it can act as a neurotransmitter related to memory. In a study made on rats, it was found out that L-theanine modulated the serotonin and dopamine levels and increased learning skills with memory (Unno et al., 1999). In addition, it is stated that L-theanine increases neurothrophine mRNA level by activating its neurotransmitter inhibiting system and supports the central nervous system that helps the development of brain functions (Yamada et al., 2007). When the linear electrocardiographic records with cognitive task performance were examined, it was found out that L-theanine was the only amino acid that works synergetic with caffeine in increasing the attention process and cognitive performance in humans (Kelly et al., 2008). There are some mechanisms explaining those effects. First of all, it is stated that L-theanine exceeds blood-brain barrier and shows cerebroprotective effect. In addition, it shows preventive effect on neuronal cell death after transient cerebral ischemia. That neuroprotective effect is partly caused by the antagonist effect of glutamate receptor on its subtype AMPA and kainate receptors. However, its affinity to those receptors is not much. Besides, L-theanine acts as if it is glutamine carrier and inhibits combination of extra cellular glutamine with neurons (Kakuda, 2011).

The effect of L-theanine on cognitive performance when given with caffeine is another topic of discussion. In a randomized, placebo-controlled double blind study, the effect of caffeine (150 mg) and L-theanine (250 mg) alone or in combination on cognitive performance and emotional state was examined. In the end, it was found out that semantic memory was more sensitive to caffeine when compared to L-theanine (Haskell et al., 2008). In a metaanalysis study, in which 11 randomized placebo-controlled studies on humans were gathered, the effect of L-theanine alone or in combination with caffeine given to the human on the cognitive performance and emotional state was examined. Two hours after taking the L-theanine and caffeine combination attention and emotional state outputs in a lower level were affected and similar changes were observed after one hour mostly with caffeine + L-theanine combination, less with caffeine alone and the least with L-theanine alone (Camfield et al., 2014).

Because of the effects of L-theanine on the cognitive performance, it has made the elderly individuals, with whom especially decreases in cognitive performance is frequently observed, its focus point. For example, in a study conducted on individuals with normal or slight cognitive performance dysfunction and who were given a dose of green tea powder with high L-theanine concentration (47.5 mg/day), it was found out that the decreases in cognitive function in the group, who were given green tea powder with high L-theanine concentration, was considerably less than the placebo group (Kakuda, 2011). Again, because it was one of the most important pathologic mechanisms of toxicity Alzheimer disease which Amyloid β (Aβ) causes, the inhibiting effect of L-theanine, which is a component of *Camellia sinensis* (green tea), on neuronal cell death caused by Aβ1–42 and on cognitive dysfunction was examined. In the study, after Aβ1–42 was given through intracerebra ventricular (2 μg), 2 and 4 mg/kg doses of L-theanine was added to the drinking waters of mice for a period of five weeks. At the end of the study, L-theanine decreased the level of Aβ1–42 and also the neuronal cell death accompanying to Aβ1–42 in the cortex and hippocampus of the brain. In addition, L-theanine inhibited activities of extracellular signal regulator kinase (ERK) caused by Aβ1–42 and mitogen activating protein kinase (MAPK) p38 together with nuclear factor kappa b (NF-κB). At the same time, L-theanine decreased the protein and lipid oxidation and increased the glutathione level in the brain. Those results made us think that L-theanine had a positive effect on memory and had positive effects on the treatment and prevention of Alzheimer (Kim et al., 2009).

L-theanine and sleep

Because of its effects on emotional state and stress, it is thought that L-theanine may also have effect on sleep and some studies have been conducted on that subject. However, the studies on that subject are very limited. In a study, where the effect of L-theanine on the sleep quality of 98 boys (8–12 years old) with attention deficit and hyperactivity disorder was examined, the children were given chewable L-theanine tablets (a total of 400 g/day) or placebo two times a day. At the end of the study, sleep percentage and efficiency scores of the boys who were given L-theanine were higher, but their tendencies for keeping less awake after the beginning of sleep were the same. There were no changes in sleep latency, which is a part of sleep quality components, and sleep parameters. As a result, it was reported that L-theanine partly increased the sleep quality at the children with attention deficit and hyperactivity disorder; however, it should be supported with further studies (Lyon et al., 2011).

It is stated that L-theanine may inhibit the stimulant effect of caffeine on sleep problems. In a study, rats were given caffeine and L-theanine in various doses of 22.5, 37.5, 75, or 150 mg/kg and it was found out that not the doses of 75 and 150 mg/kg, but the doses of 22.5 and 37.5 mg/kg did not decrease the wakefulness stimulated by caffeine. However, it was found out that L-theanine partly reversed the decreases in slow sleep wave (Jang et al., 2012).

L-theanine and cancer

It is thought that L-theanine has a role in preventing cancer as well. It was found out in a study that L-theanine in vivo and in vitro inhibited the growth of lung cancer and leukemia cells (Liu et al., 2009). In another study, it was found out that L-theanine stimulated the apoptosis of breast cancer, colon cancer, liver, and prostate cancer cells (Friedman et al., 2007).

Effect of L-theanine on antitumor drugs is the most focused subject in experimental studies. In the studies conducted, it was found out that L-theanine inhibited the leakage out f tumor cells of an antitumor drug called doxorubicin and increased the concentration in tumor cells (Sugiyama and Sadzuka, 2004; Friedman et al., 2007; Liu et al., 2009). In addition, it enables the transport of the drug to tumor cells, increases the efficiency and may decrease the resistance against the drug. Those effects occur as L-theanine inhibits the glutamate carriers, inhibits the glutathione synthesis from glutamate and decreases glutathione doxorubicin concentration and leakage outside through MRP/GS-X pump. It is reported that it causes significant increases in antitumor activities in those ways (Sugiyama and Sadzuka, 2004; Liu et al., 2009; Zhang et al., 2013). Besides, it is claimed that L-theanine may decrease the hepatotoxicity that occurs as the result of the toxicity of antitumor drugs such as doxorubicin, renal functional disorder, and negative changes seen in hematopoetic system. That is said to be related with the fact that the increasing effect of L-theanine on the deposition increasing, limited with the tumor cell and it does not have such effect on normal tissues such as liver, lung, and kidneys (Sugiyama and Sadzuka, 2004; Liu et al., 2009; Zhang et al., 2013). In a study conducted, it was found out that L-theanine given together with a 10 mg/kg dose of doxorubicin suppressed serum lactate dehydrogenase and creatine kinase activities, thus it may prevent the cardiac acute damage caused by doxorubicin. In the same study, it was also found out that L-theanine suppressed the body weight loss caused by doxorubicin as well (Jang et al., 2012). In addition, there are some studies that report that L-theanine prevents the metastasis of tumor cells

(Nagai and Konishi, 2013; Zhang et al., 2013). For example, in a study conducted, it was reported that L-theanine, which was given together with doxorubicin, adriamisin with its commercial name, suppressed the hepatic metastasis of cells with over sarcoma (Nagai and Konishi, 2013).

L-theanine and cardiovascular diseases

Tea is generally related with the cardiovascular diseases (Nantz et al., 2009). And L-theanine, an amino acid special to tea, is generally related with hypertension, one of the risk factors of cardiovascular diseases, in animal and culture studies. In a study conducted, it was found out that, more in the hypertensive rats than the self-normotensive rats, L-theanine decreased the 5-hydroxindol level and blood pressure in the brain (Yokogoshi et al., 1995). In another culture study, it was found out that it caused relaxation in the vascular smooth muscle cells when given as a mixture with epigallocatechin gallate (Yokozawa et al., 1995). It is reported that the hypotensive effect of L-theanine depends on the dose.

One of the other hypotensive effects of L-theanine is to change the neurotransmitter level in the brain. Again, it is stated that L-theanine increases the alpha activity in occipital and parietal cortexes and creates relaxation and that may cause decrease in the blood pressure (Kimura et al., 2007).

There are studies in the literature that suggest tea consumption develops the vascular function and decreases cardiovascular diseases. By increasing the production of vascular nitric oxide (NO) in the endothelium in the cells, L-theanine supports that hypothesis. Because, endothelial NO acts as the main regulator of vascular function. In an in vitro study that supports this, it was reported that L-theanine application increased the NO production through ERK/eNOS activation and vasodilatation developed in the arteries in connection with that (Siamwala et al., 2013).

Effect of L-theanine on the low density lipoprotein (LDL) oxidation that has a significant contribution to atherosclerosis is another topic that is studied on. In the studies conducted, it has been reported that like tea flavanoids, L-theanine decreased LDL oxidation as well (Yokozawa and Dong, 1997; Fraser et al., 2007). However, it is reported that flavanoids are the most responsible for the delay of peroxidation caused in relation with the dose related effect of LDL peroxidation to green tea extract. It is stated that L-Theanine contributes to this delay less than caffeine and more than flavanoids (Yokozawa and Dong, 1997).

L-theanine and food intake-obesity

The studies on the effect of L-theanine on food intake and obesity are very few and they are limited with studies on animals (Sayama et al., 1999; Yamada et al., 2008). Some studies support that L-theanine affects the food intake by changing the hormone levels. In a study, where the role of L-theanine in food intake is examined and supports that thesis, it was found out that, when taken orally, L-theanine suppressed food intake in rats, it caused no change in glucose level, but caused a significant decrease in insulin concentration and a significant increase in corticosterone level (Yamada et al., 2008). In another study, it was suggested that L-theanine changed the dopamine and serotonin neurotransmission in brain and affected food intake (Sayama et al., 1999).

There are few studies examining the relation between L-theanine and obesity in literature (Zheng et al., 2004). In a study, the antiobesity effects of three main components of green tea, catechin, caffeine and L-theanine, were examined. In that frame, female mice were given a diet, which was composed of 2% green tea powder, 0.3% catechin, 0.05% caffeine, and 0.03% L-theanine, or fed with combinations made of 2% green tea powder for 16 weeks. During that period, changes in body weight and food intake together with changes of intraperitoneal adipose tissue weight were recorded monthly. At the end of the study, intraperitoneal adipose tissue mass of the mice fed with the diet composed of green tea, caffeine, L-theanine, caffeine + catechin, and caffeine + catechin + L-theanine. There was 76.8% more loss in the intraperitoneal adipose tissue in the group fed with caffeine + catechin diet than the control group. However, it was found out that green tea suppressed body weight and fat deposition preventive effect when especially L-theanine or caffeine was given with green tea. At the same time, it was found out in the study that the food intake of the mice fed with L-theanine decreased in a nonsignificant level (Zheng et al., 2004).

L-theanine and common cold

The positive effect of L-theanine on flu and common cold has become forward recently. In general, the combinations with other bioactive components have effect on common cold. In a randomized double blind and placebo-controlled study conducted on health workers, the individuals were given a dose of 378 mg/day green tea catechin and 210 mg/day L-theanine for a period of five months. At the end, the clinically proven flu infection incidence of the group that took catechin/L-theanine group (4.1%) was found out much lower than the placebo group (13.1%). In the light of those results, it was reported that catechin/L-theanine might be used for prophylactic purposes for the prevention of flu infection (Matsumoto et al., 2011). In another randomized double blind and placebo-controlled study conducted, 176 male individuals were given placebo or L-cysteine and L-theanine mixture capsules (490 mg) two times a day for 35 days. At the end, the common cold incidence in the group given L-cysteine and L-theanine mixture capsules was seen lower compared to the placebo group and the sickness period was the same for both groups (Kurihara et al., 2010). In a study conducted on the mice, L-cysteine and L-theanine incited the production of antibodies specific to antigen after antigen stimulation. Those effects are caused because it increases the glutathione synthesis and humoral immune response (Kurihara et al., 2007). That immune response is generally linked with the increase of $\gamma\delta$T lymphocyte function (Bukowski and Percival, 2008).

Safety of L-theanine

Taking high dose intake of L-theanine with diet is reported to be safe. Food and Drug Agency (FDA) states that the estimated daily intake amount of L-theanine with diet is

628 mg per person and 90th percentile value of intake is 1284 mg/day per person. So, in 2011 FDA suggests that daily consumption amount of L-theanine should not exceed 1200 mg (FDA, 2006; Vuong et al., 2011). As the result of acute and subacute toxicity and mutagenity tests conducted by Japan Food Additives Association Suntheanine has been accepted as reliable. Currently, no dietetic exposure limits have been suggested for L-theanine by Japan Food Additives Association (Juneja et al., 1999).

It is stated that L-theanine has not shown toxic effect either on animals or on humans. For example, in a subchronic toxicity study conducted on the rodents, it was stated that taking 4.000 mg L-theanine per body weight for 90 days was safe, in general, and the kidney lesions occurred on three rodents might rather be related with genetic disposition than with direct toxic effect (FDA, 2006). Again, it was determined in the pathologic studies conducted that the pathologic effects related with L-theanine were not related with the dose. In a study that examined the effect of L-theanine depending on the dose, L-theanine (Suntheanine) was given to female and male rats in 0, 1500, 3000, or 4000 mg/kg body weight doses for 13 weeks and it was reported that it did not cause any negative effect at pathologic, organ weight, or histopathologic levels (Borzelleca et al., 2006). Again in another study, after orally giving L-theanine diet in 0–5% concentrations (maximum tolerable dose) to B6C3F1 rats, subacute test for the 13 weeks and chronic toxicity test for the following 78 weeks were conducted. No difference was reported between intervention and control groups in terms of diet intakes, weight gains, or survival rates. However, there was significant decrease in the L-theanine applied group at the end of 78 weeks in terms of tumor incidence and the total number of tumors. At the end, it was shown that long-term L-theanine oral application to rats did not cause chronic toxicological or tumorigenic aberrations (Fujii and Inai, 2008).

Conclusions and recommendations

L-theanine amino acid can be regarded as a bioactive component which has been begun to be considered recently both for its metabolic process and its possible health effects. Especially the studies conducted on its health effects are mostly limited with animal studies and they are limited in terms of human studies. EFSA (2011) stated that, the cause and effect relation between *Camellia Sinensis* (L.) Kuntze (tea) sourced L-theanine consumption and cognitive function, decrease in physiologic stress, continuation of sleep in a normal manner could not be accepted because of inadequate number or lack of studies in that field (EFSA, 2011). Within that frame, there is a need for more studies on the possible health effects of L-theanine. While studying on that effect, the effects of various doses, forms (in synthetic or natural product matrix), and exposure in different periods (short or long term) on health should be studied. However, currently, the conducted studies are promising for: L-theanine is a bioactive component like catechin and caffeine. In addition, although the fact intake with diet proved to be reliable at the end of the conducted acute and chronic toxicity test is another positive part, safety of L-theanine should be supported through further studies.

References

Alcázar, A., Ballesteros, O., Jurado, J. M., Pablos, F., Martín, M. J., Vilches, J. L. and Navalón, A. (2007). Differentiation of green, white, black, Oolong, and Pu-erh teas according to their free amino acids content. *J. Agric. Food Chem.* **55**(15):5960–5965.

Anonymous. (2013). World Tea News Market Reports: Increasing Value for the World's Most Consumed Beverage. Issue: 4.

Borzelleca, J., Peters, D. and Hall, W. (2006). A 13-week dietary toxicity and toxicokinetic study with L-theanine in rats. *Food Chem. Toxicol.* **44**(7):1158–1166.

Bryan, J. (2008). Psychological effects of dietary components of tea: Caffeine and L-theanine. *Nutr. Rev.* **66**(2):82–90.

Bukowski, J. F. and Percival, S. S. (2008). L-theanine intervention enhances human $\gamma\delta$T lymphocyte function. *Nutr. Rev.* **66**(2):96–102.

Camfield, D. A., Stough, C., Farrimond, J. and Scholey, A. B. (2014). Acute effects of tea constituents L-theanine, caffeine, and epigallocatechin gallate on cognitive function and mood: A systematic review and meta-analysis. *Nutr. Rev.* **72**(8):507–522.

Chu, D. C. (1997). Green tea: Its cultivation, processing of the leaves for drinking materials, and kinds of green tea. **In**: Chemistry and Applications of Green Tea, pp. 1–11. Yamamoto, T., Lekh, J. R., Chu, D. C. and Kim, M., Eds., CRC Press, Boca Raton.

da Silva Pinto, M. (2013). Tea: A new perspective on health benefits. *Food Res. Int.* **53**(2):558–567.

Deng, W. W., Ogita, S. and Ashihara, H. (2008). Biosynthesis of theanine (γ-ethylamino-l-glutamic acid) in seedlings of Camellia sinensis. *Phytochem. Lett.* **1**(2):115–119.

Desai, M. J., Gill, M. S., Hsu, W. H. and Armstrong, D. W. (2005). Pharmacokinetics of theanine enantiomers in rats. *Chirality.* **17**(3):154–162.

Ekborg-Ott, K. H., Taylor, A. and Armstrong, D. W. (1997). Varietal differences in the total and enantiomeric composition of theanine in tea. *J. Agric. Food Chem.* **45**(2):353–363.

European Food Safety Authority (EFSA). (2011). Panel on Dietetic Products, Nutrition and Allergies (NDA): Scientific opinion on the substantiation of health claims related to L-theanine from Camellia sinensis (L.) Kuntze (tea) and improvement of cognitive function (ID 1104, 1222, 1600, 1601, 1707, 1935, 2004, 2005), alleviation of psychological stress (ID 1598, 1601), maintenance of normal sleep (ID 1222, 1737, 2004), and reduction of menstrual discomfort (ID 1599) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA J.* **9**(6):223–228.

Food and Drug Administration (FDA) CFSAN/Office of Food Additive Safety. Agency Response Letter GRAS Notice No. GRN 000209. GRAS Notice Inventory of L-Theanine, 2006.

Fraser, M. L., Mok, G. S. and Lee, A. H. (2007). Green tea and stroke prevention: Emerging evidence. *Complement. Ther. Med.* **15**(1):46–53.

Friedman, M., Mackey, B. E., Kim, H. J., Lee, I. S., Lee, K. R., Lee, S. U., Kozukue, E. and Kozukue, N. (2007). Structure-activity relationships of tea compounds against human cancer cells. *J. Agric. Food Chem.* **55** (2):243–253.

Fujii, S. and Inai, K. (2008). Tumorigenicity study of L-theanine administrated orally to mice. *Food Chem.* **110**(3):643–646.

Gomez-Ramirez, M., Higgins, B. A., Rycroft, J. A., Owen, G. N., Mahoney, J., Shpaner, M. and Foxe, J. J. (2007). The deployment of intersensory selective attention: A high-density electrical mapping study of the effects of theanine. *Clin. Neuropharmacol.* **30**(1):25–38.

Haskell, C. F., Kennedy, D. O., Milne, A. L., Wesnes, K. A. and Scholey, A. B. (2008). The effects of L-theanine, caffeine, and their combination on cognition and mood. *Biol. Psychol.* **77**(2):113–122.

Hilal, Y. and Engelhardt, U. (2007). Characterization of white tea: Comparison to green and black tea. *J. Verbr. Lebensm.* **2**(4):414–421.

Jain, A., Manghani, C., Kohli, S., Nigam, D. and Rani, V. (2013). Tea and human health: The dark shadows. *Toxicol. Lett.* **220**(1):82–87.

Jang, H. S., Jung, J. Y., Jang, I. S., Jang, K. H., Kim, S. H., Ha, J. H., Suk, K. and Lee, M. G. (2012). L-theanine partially counteracts caffeineinduced sleep disturbances in rats. *Pharmacol. Biochem. Behav.* **101**(2):217–221.

Juneja, L. R., Chu, D. C., Okubo, T., Nagato, Y. and Yokogoshi, H. (1999). L-theanine: A unique amino acid of green tea and its relaxation effect in humans. *Trend Food Sci. Technol.* **10**(12):425.

Kakuda, T. (2011). Neuroprotective effects of theanine and its preventive effects on cognitive dysfunction. *Pharmacol. Res.* **64**(2):162–168.

Keenan, E. K., Finnie, M. D. A., Jones, P. S., Rogers, P. J. and Priestley, C. M. (2011). How much theanine in a cup of tea? Effects of tea type and method of preparation. *Food Chem.* **125**(2):588–594.

Kelly, S. P., Gomez-Ramirez, M., Montesi, J. L. and Foxe, J. J. (2008). L-theanine and caffeine in combination affect human cognition as evidenced by oscillatory alpha-band activity and attention task performance. *J. Nutr.* **138**(8):1572–1577.

Kim, T. I., Lee, Y. K., Park, S. G., Choi, I. S., Ban, J. O., Park, H. K., Nam, S. Y., Yun, Y. W., Han, S. B., Oh, K. W. and Hong, J. T. (2009). L-theanine, an amino acid in green tea, attenuates β-amyloid-induced cognitive dysfunction and neurotoxicity: Reduction in oxidative damage and inactivation of ERK/p38 kinase and NF-κB pathways. *Free Radic. Biol. Med.* **47**(11):1601–1610.

Kimura, K., Ozeki, M., Juneja, L. R. and Ohira, H. (2007). L-theanine reduces psychological and physiological stress responses. *Biol. Psychol.* **74**(1):39–45.

Kobayashi, K., Nagato, Y., Aoi, N., Juneja, L., Kim, M., Yamamoto, T. and Sugimoto, S. (1998). Effects of L-theanine on the release of alpha-brain waves in human volunteers. *Nippon Nogeik. Kaishi* **72**(2):153–157.

Kobayashi, M. and Ikeda, I. (2014). Chapter 48: Modulation of intestinal cholesterol absorption by dietary tea polyphenols. **In**: Polyphenols in Human Health and Disease, pp. 625–638. Watson, R. R., Preedy, V. R. and Zibadi, S., Eds., Academic Press, San Diego.

Kurihara, S., Hiraoka, T., Akutsu, M., Sukegawa, E., Bannai, M. and Shibahara, S. (2010). Effects of L-cystine and L-theanine supplementation on the common cold: A randomized, double-blind, and placebo-controlled trial. *J. Amino Acids* **2010**:1–7.

Kurihara, S., Shibahara, S., Arisaka, H. and Akiyama, Y. (2007). Enhancement of antigen-specific immunoglobulin G production in mice by coadministration of L-cystine and L-theanine. *J. Vet. Med. Sci.* **69**(12):1263–1270.

Lin, Y. S., Tsai, Y. J., Tsay, J. S. and Lin, J. K. (2003). Factors affecting the levels of tea polyphenols and caffeine in tea leaves. *J. Agric. Food Chem.* **51**(7):1864–1873.

Liu, Q., Duan, H., Luan, J., Yagasaki, K. and Zhang, G. (2009). Effects of theanine on growth of human lung cancer and leukemia cells as well as migration and invasion of human lung cancer cells. *Cytotechnology.* **59**(3):211–217.

Lu, K., Gray, M. A., Oliver, C., Liley, D. T., Harrison, B. J., Bartholomeusz, C. F., Phan, K. L. and Nathan, P. J. (2004). The acute effects of L-theanine in comparison with alprazolam on anticipatory anxiety in humans. *Human Psychopharmacol.* **19**(7):457–465.

Lyon, M. R., Kapoor, M. P. and Juneja, L. R. (2011). The effects of L-theanine (Suntheanine(R)) on objective sleep quality in boys with attention deficit hyperactivity disorder (ADHD): A randomized, double-blind, placebo-controlled clinical trial. *Altern. Med. Rev.* **16**(4):348–354.

Matsumoto, K., Yamada, H., Takuma, N., Niino, H. and Sagesaka, Y. M. (2011). Effects of green tea catechins and theanine on preventing influenza infection among healthcare workers: A randomized controlled trial. *BMC Complement. Altern. Med.* **11**(1):15.

Nagai, K. and Konishi, H. (2013). Protection of theanine against doxorubicin-induced acute cardiac toxicity. *Biomed. Prev. Nutr.* **3**(2):197–199.

Nantz, M. P., Rowe, C. A., Bukowski, J. F. and Percival, S. S. (2009). Standardized capsule of Camellia sinensis lowers cardiovascular risk factors in a randomized, double-blind, placebo-controlled study. *Nutrition (Burbank, Los Angeles County, Calif).* **25**(2):147–154.

Narukawa, M., Toda, Y., Nakagita, T., Hayashi, Y. and Misaka, T. (2014). L-theanine elicits umami taste via the T1R1 + T1R3 umami taste receptor. *Amino Acids* **46**(6):1583–1587.

Sakato, Y. (1949). The chemical constituents of tea: III. A new amide theanine. *Nippon Nogeik. Kaishi* **23**:262–267.

Sayama, K., Lin, S., Zheng, G. and Oguni, I. (1999). Effects of green tea on growth, food utilization, and lipid metabolism in mice. *In Vivo (Athens, Greece).* **14**(4):481–484.

Siamwala, J. H., Dias, P. M., Majumder, S., Joshi, M. K., Sinkar, V. P., Banerjee, G. and Chatterjee, S. (2013). L-theanine promotes nitric oxide production in endothelial cells through eNOS phosphorylation. *J. Nutr. Biochem.* **24**(3):595–605.

Song, C. H., Jung, J. H., Oh, J. S. and Kim, K. S. (2003). Effects of theanine on the release of brain alpha wave in adult males. *Korean J. Nutr.* **36**(9):918–923.

Sugiyama, T. and Sadzuka, Y. (2004). Theanine, a specific glutamate derivative in green tea, reduces the adverse reactions of doxorubicin by changing the glutathione level. *Cancer Lett.* **212**(2):177–184.

The Republic of Turkey Ministry of Health and Hacettepe University Nutrition and Dietetics Department. Turkey Nutrition and Health Survey 2010. Nutrition Situation and Behaviors Evaluation Result Report. Directorate General for Health Surveys Publication No: SB-SAG-2014/0, ISBN : 978–975–590–483–2.

Unno, T., Suzuki, Y., Kakuda, T., Hayakawa, T. and Tsuge, H. (1999). Metabolism of theanine, γ-glutamylethylamide, in rats. *J. Agric. Food Chem.* **47**(4):1593–1596.

van der Pijl, P. C., Chen, L. and Mulder, T. P. J. (2010). Human disposition of L-theanine in tea or aqueous solution. *J. Funct. Foods* **2**(4):239–244.

Vuong, Q. V., Bowyer, M. C. and Roach, P. D. (2011). L-theanine: Properties, synthesis, and isolation from tea. *J. Sci. Food Agric.* **91**(11):1931–1939.

Vuong, Q. V. (2014). Epidemiological evidence linking tea consumption to human health: A review. *Crit. Rev. Food Sci. Nutr.* **54**(4):523–536.

Yamada, T., Nishimura, Y., Sakurai, T., Terashima, T., Okubo, T., Juneja, L. R. and Yokogoshi, H. (2008). Administration of theanine, a unique amino acid in tea leaves, changed feeding-relating components in serum and feeding behavior in rats. *Biosci. Biotechnol. Biochem.* **72**(5):1352–1355.

Yamada, T., Terashima, T., Wada, K., Ueda, S., Ito, M., Okubo, T., Juneja, L. R. and Yokogoshi, H. (2007). Theanine, r-glutamylethylamide, increases neurotransmission concentrations and neurotrophin mRNA levels in the brain during lactation. *Life Sci.* **81**(16):1247–1255.

Ying, Y., Ho, J. W., Chen, Z. Y. and Wang, J. (2005). Analysis of theanine in tea leaves by HPLC with fluorescence detection. *J. Liq. Chromatogr. R. T.* **28**(5):727–737.

Yokogoshi, H., Kato, Y., Sagesaka, Y. M., Takihara-Matsuura, T., Kakuda, T. and Takeuchi, N. (1995). Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats. *Biosci. Biotechnol. Biochem.* **59**(4):615–618.

Yokozawa, T. and Dong, E. (1997). Influence of green tea and its three major components upon low-density lipoprotein oxidation. *Exp. Toxicol. Pathol.* **49**(5):329–335.

Yokozawa, T., Oura, H., Nakagawa, H., Sakanaka, S. and Kim, M. (1995). Effects of a component of green tea on the proliferation of vascular smooth muscle cells. *Biosci. Biotechnol. Biochem.* **59**(11):2134–2136.

Yoto, A., Motoki, M., Murao, S. and Yokogoshi, H. (2012). Effects of L-theanine or caffeine intake on changes in blood pressure under physical and psychological stresses. *J. Physiol. Anthropol.* **31**:28.

Zhang, L., Zhang, Z. Z., Lu, Y. N., Zhang, J. S. and Preedy, V. R. (2013). Chapter 35: L-theanine from green tea: Transport and effects on health. **In**: Tea in Health and Disease Prevention, pp. 425–435. Preedy, V. R., Ed., Academic Press, USA.

Zhao, M., Ma, Y., Wei, Z. Z., Yuan, W. X., Li, Y. L., Zhang, C. H., Xue, X. T. and Zhou, H. J. (2011). Determination and comparison of gamma-aminobutyric acid (GABA) content in pu-erh and other types of Chinese tea. *J. Agric. Food Chem.* **59**(8):3641–3648.

Zheng, G., Sayama, K., Okubo, T., Juneja, L. and Ogunı, I. (2004). Antiobesity effects of three major components of green tea, catechins, caffeine, and theanine, in mice. *In Vivo* **18**(1):55–62.