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How much theanine in a cup of tea? Effects of tea type and method of preparation

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ABSTRACT

Recent interest into the possible benefits of L-theanine found in tea has raised the issue that there are few data available on amounts of L-theanine contained in cups of commercially-available teas, prepared by a standard method. HPLC along with a standard method of preparing tea was employed here to determine amounts of L-theanine in cups of tea and the effects that various preparation factors have on amounts of L-theanine extracted. Brewing time was found to be a major determinant of the amount of L-theanine extracted, while the addition of small amounts of milk and sugar made no significant difference. High levels of milk resulted in a marked lowering of the level of detectable L-theanine. Contrary to previous research, a standard (200 ml) cup of black tea was found to contain the most L-theanine (24.2 ± 5.7 mg) while a cup of green tea contained the least (7.9 ± 3.8 mg).

1. Introduction

L-Theanine (γ-glutamylethylamide, CAS No. [3081-61-6]) is a non-proteinogenic amino acid found in tea (its primary dietary source) and a derivative of the amino acid glutamic acid. Tea is the most widely consumed beverage worldwide after water and is generally seen as a relaxing drink, whereas coffee is seen as more of an energising drink (Rogers, Smith, Heatherley, & Pleydell-Pearce, 2008). Although both drinks contain caffeine, L-theanine is only present in tea. This difference has led to an interest in the effects of L-theanine on psychological measures, such as stress and anxiety. L-Theanine has been found to have a relaxing effect (Juneja, Chu, Okubo, Nagato, & Yokogoshi, 1999) and to increase alpha brain waves, synonymous with a relaxed yet alert state (Nobre, Rao & Owen, 2008). Recent research has found that L-theanine reduced both subjective and physiological stress responses during a stressful task situation (Kimura, Ozeki, Juneja, & Ohira, 2007), and that it may be useful for reducing raised blood pressure (Rogers et al., 2008). This relaxation effect of L-theanine may also explain the improvements seen in subjective sleep quality and mood upon awakening after L-theanine (Ozeki, Juneja, & Shirakawa, 2003). This interest in beneficial effects of L-theanine has raised the question of what quantities of L-theanine are contained in various commercially-available teas. Such data are essential for calculation of average daily consumption of L-theanine by tea consumers, similar to the reports for caffeine consumption (Rogers et al., 2008).

Previous studies have analysed L-theanine amounts per gram of tea leaf (Alcazar et al., 2007; Syu, Lin, Huang, & Lin, 2008; Thippeswamy et al., 2006) or as a percentage (Hilal and Engelhardt, 2007; Ying et al., 2005), and amounts at different stages of the growing (Feldheim, Yongvanit, & Cummings, 1986) and manufacturing process (Ying et al., 2005). L-Theanine has been found to accumulate more in young and active tissues and in younger plants (Feldheim et al., 1986; Horie & Kohata, 1998), and to vary between 2 and 5 mg/g leaf (Thippeswamy et al., 2006). The highest quality black teas have been found to contain the lowest amounts of L-theanine (Feldheim et al., 1986; Ying et al., 2005). Apart from the older leaves, the first leaves of a matured tea bush were found to contain the lowest concentrations of L-theanine, which was linked to the biosynthesis of polyphenols, since they are concentrated mostly in these leaves (Feldheim et al., 1986).

There are three major categories of tea, which are defined by their fermentation process. Green and white teas are unfermented, black tea is fully fermented and oolong teas are half fermented. In the literature the extent of fermentation has been found to be a determinant of the concentration of L-theanine, with more L-theanine contained in unfermented green teas (Alcazar et al., 2007; Ying et al., 2005) and the most L-theanine contained in white teas

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(Alcazar et al., 2007; Hilal & Engelhardt, 2007). There is no agreed definition for white tea. Chinese white tea is defined by the subspecies from which it is manufactured. Other countries that produce white tea define it by the plucking standard, using only the buds and young leaves of the tea plant (Hilal & Engelhardt, 2007). However, both undergo minimal processing with no fermentation. For practical applications, L-theanine amounts per standard cup of commercially-available teas would be very useful for psychometric studies. Most commercial teas are different blends of various qualities of tea sourced from different parts of the world.

There are however also large variations in the way that tea is prepared by the consumer. Variations include the vessel (cup or pot) in which the tea is prepared, how much water is used and its temperature, how long the tea or bag is left to infuse and whether the tea bag is dunked or stirred, and left in or removed. Tea is also often combined with sugar or milk. Studies of the caffeine and polyphenols content of various teas and preparations have found that factors such as brew time (Astill, Birch, Dacombe, Humphrey, & Martine, 2001; Kyle, Morrice, McNeill, & Duthie, 2007), stirring, and whether the tea is in loose leaf or bag form are important determinants of the amounts of caffeine and polyphenols extracted (Astill et al., 2001). These studies highlight the need to collect information on preparation methods and tea type, as well as frequency of consumption, to arrive at a reliable estimate of caffeine exposure. It is reasonable to assume that these preparation factors would also affect the amounts of L-theanine contained in a cup of tea and that the same factors apply to the assessment of L-theanine consumption.

A standard preparation method of brewing tea needed to be devised so that deviations could be determined for the more dynamic variables that affect the extraction of L-theanine. Hilal and Engelhardt (2007) estimated that a standard 200-ml cup would contain around 10–20 mg/l theanine. A recent review of L-theanine by Bryan (2008) deduced from earlier studies that L-theanine constitutes between 1–2% of the dry weight of tea (Finger, Kuhr, & Engelhardt, 1992) and estimated that this would equate to around 25–60 mg L-theanine per 200-ml serving. This is about the amount of liquid contained in a cup of tea and also forms part of the recommended brewing instructions for PG Tips (a brand of black tea), so was adopted in this study as the standard measure.

According to consumer studies referred to by Astill et al. (2001), brew times vary from fewer than 30 s to greater than five minutes, with an average brew time of fewer than 2 min. Packet instructions vary in their recommendations for brewing time from 1 to 5 min. Previous studies have used brew times between 10 and 30 min (Alcazar et al., 2007; Syu et al., 2008; Thippeswamy et al., 2006; Ying et al., 2005), which may not provide a realistic assessment of the amount of L-theanine contained in a cup of tea brewed in the home. A brew time of 2 min was used as the standard here, although the effects of brew time were also considered.

Astill et al. (2001) referred to stirring and 'dunking' of the tea bag as 'mechanical agitation' and found that it was an important determinant of the amounts of caffeine and polyphenols extracted. They also found that this was not the case for loose-leaf teas, for which the concentrations of tea components recorded were independent of the amount of agitation used. This is a difficult variable to account for as the tea bag may be stirred, dunked or both, and may only be agitated at the beginning or end of infusion or for the total infusion time. If the tea bag is to be removed from the vessel used it is likely that it will be squeezed against the side of the vessel to remove any excess liquid. This forms part of the standard method used here, although the amount of force used in squeezing the bag against the side of the vessel was likely to be another factor that affects the amount of L-theanine released into the sample.

According to The Tea Appreciation Society 98% of people take milk in their tea and the most commonly used milk in the UK is semi-skimmed. In an evaluation of how to make the perfect cup of tea, Tetley recommend 16 ml milk in a 270-ml cup of tea (12 ml per 200-ml cup). Therefore, different amounts of semi-skimmed milk (5, 12 & 50 ml), and skimmed and full-fat milk (12 ml) were added to tea, to assess the effects that quantity and type of milk may have on L-theanine content. The possible effects of added sugar were also assessed, as 45% people in the UK take sugar in their tea (The Tea Appreciation Society). Factors such as vessel type and amount of water used were not assessed in this study. A discussion of these brewing variations is available in Astill et al. (2001) and although their paper describes brewing methods that affect caffeine, similar effects might be expected for L-theanine.

The primary purpose of this report is to provide the most comprehensive assessment to date of the amounts of L-theanine per 200-ml cup of various popular commercial tea products in the UK. Because, to our knowledge, no other studies have investigated whether brewing time or the addition of milk or sugar affect the amount of L-theanine released, this report also covers an initial assessment of these factors, which can be expanded in future research.

2. Materials and methods

2.1. Chemical and Reagents

L-Theanine (CAS No [3081-61-6]) standard was obtained from Sigma–Aldrich (Gillingham, Dorset, UK). Acetonitrile (MeCN) and orthophosphoric acid 85% (H_3PO_4) were HPLC grade and purchased from BDH Prolabo Chemicals (Loughborough, UK). Sodium dodecyl sulphate (SDS) was obtained from National Diagnostics (Hessle, UK). All solutions were prepared with de-ionised water (Millipore, Watford, UK).

2.2. The method for analysis of L-theanine by reverse-phase HPLC

HPLC analysis was performed on an Agilent 1100 HPLC system equipped with a diode array detector (DAD) and a Luna C18 (2), 150 × 4.6 mm, 5-μm particle size column with a C18 guard cartridge. Mobile phase A was prepared fresh for every run and consisted of 10 mM SDS in 65% de-ionised water, 35% MeCN and 0.1% H_3PO_4. Mobile phase B was 5% MeCN. The column was eluted with 100% A for 1.5 min, then eluted with 5% B on a linear gradient increasing to 95% B over 15 min. The elution was then returned to 5% B over 3 min and allowed to re-equilibrate for 10 min. The mobile phase was delivered at a constant flow rate of 1.0 ml/min. The analytes were monitored by UV detection at 205 ± 4 nm. Injection volume was 10 μl.

2.3. HPLC method calibration

The accuracy of the reverse-phase HPLC method was assessed by recovery experiments. The stock standard was prepared by sonicating 7.56 mg L-theanine in 8 ml de-ionised water in a 10-ml volumetric flask for 15 min. After re-equilibrating to ambient temperature, this was diluted to volume with de-ionised water and a series of five dilutions covering the range 100–500 mg/l were prepared for calculation of a calibration curve. Recovery percentages were calculated by comparing the measured L-theanine to the added L-theanine. A separate calibration curve was determined for each run and a check standard (600 mg/l) included, allowing calculation of recovery. Stock standard solutions were prepared fresh for every run.

2.4. Tea samples

Twenty-seven popular commercial teas (PG Tips, Tetley, Typhoo, Yorkshire Tea, Café Direct, Twinings and Clipper) and super-

market own-brand teas were purchased from three large supermarkets in the UK (Tesco, Sainsbury's and Asda). These included seventeen black teas (of which one was decaffeinated, one was a loose-leaf tea and one was granulated), five Twinings speciality black teas (Assam, Earl Grey, Ceylon, Lady Grey and English Breakfast), one Twinings White Tea and three green tea samples (including one loose-leaf green tea). Redbush tea originates from a different plant species (*Aspalathus linearis*) and was also assessed for L-theanine content.

2.5. Standard method of tea preparation

Freshly boiled de-ionised water (200 ml) was added to a beaker containing the tea sample and left to infuse for 2 min. The water cooled rapidly to 80 °C after being transferred to a cold beaker; therefore this was used as a standard temperature for infusion of the teas. At the end of the brewing time, the tea bag was removed from the liquid and squeezed against the side of the beaker to remove excess liquid. The preparation was then stirred to obtain an even mixture and a disposable plastic pipette was used to transfer 2 ml of the sample to an HPLC vial. Variations on this standard method for individual experiments are described in Table 1.

Tea samples were prepared using the standard method with the following exceptions. White tea was brewed at both 1 and 2 min (1 min is the manufacturer's suggested infusion time). Loose tea (one teaspoon = 2 g) was prepared in a similar way to bagged tea, except to ensure an even preparation, the tea was stirred briefly (≈3 s) at the beginning of infusion and again, after 2 min, immediately before sampling. Samples were filtered with a 45-μm PTFE disposable syringe filter, to ensure no tea leaves were transferred to the HPLC vial. Tea samples using granules (one teaspoon = 0.8 g) were prepared in a similar way except that they were stirred at the beginning to ensure all the granules dissolved in the 2 min brewing time. Samples were taken in triplicate.

Table 1
Experiments undertaken with variations on the standard method described.

Experiment	Sample used	Variant of method
1. Hot *vs* cold water	Sigma (500.8 mg) [1]	Temperature of water (12 or 80 °C)
2. Brewing time	3 PG Tips tea bags in 600 ml water [1]	Brew time (0, 0.25, 0.75, 1, 2, 5, 10, 20, 25, 30, 35, 40, 50, 60 min). Either allowed to cool or kept at a constant 80 °C. Tea bags were left in the preparation, similar to the method for making a pot of tea
3. pH levels	1 PG Tips bag in 200 ml water [1]	Starting pH levels of 3, 7 & 9
4. Sugar	1 PG Tips bag in 200 ml water [3]	One spoonful of sugar (4.2 g) or none, added after the tea bag was removed and mechanically stirred until dissolved
5. Milk type and amount	1 PG Tips bag in 200 ml water [3]	12 ml milk (none, skimmed, semi-skimmed, full fat); semi-skimmed (0, 5, 12, 50 ml) added after the tea bag was removed and mechanically stirred until an even consistency was achieved
6. Agitation methods	1 PG Tips bag in 200 ml water [3]	Water added to tea bag or tea bag to water and tea bag squeezed against the side of the beaker or not upon removal
7. Brand variations	Various [3]	Standard method
8. Brewing time of green and black tea	3 PG Tips tea bags or 3 Twinings Green tea bags in 600 ml water [1]	Brew time (0, 0.25, 0.75, 1, 2, 3, 4, 5, 10, 20, 25, 30 min). Tea bags were left in the preparation, similar to the method for making a pot of tea

Note. Number of replications is given in square brackets after the sample used.

2.6. Data analysis

Data were subjected to statistical analysis using SPSS (Version 16.0, SPSS Inc., Chicago, IL). An independent samples *t*-test was used to determine differences in L-theanine extracted in tea with and without sugar and for differences between caffeinated and decaffeinated PG Tips pyramid bag samples. For milk, one-way analysis of variance (ANOVA) was undertaken using fat content or amount as independent variables. A two-way ANOVA assessed differences in agitation method and water or tea bag in first as independent variables. To examine differences in amounts of L-theanine extracted between different types of teas, a one-way independent ANOVA with Hochberg's GT2 *post hoc* test for unequal sample sizes was used.

3. Results and discussion

3.1. Validation of the method

The initial calibration curve for varying amounts of L-theanine standard had a correlation coefficient of 0.99997. Correlation coefficients of these standard curves ranged between 0.9986 and 0.9999. Recovery percentages ranged between 98.8% and 100.%. The mean retention time of L-theanine was 4.74 ± 0.75 min.

3.2. Hot vs cold water

In Experiment 1 the amount of L-theanine contained in the sample extracted with hot water was 500.3 mg; when extracted with cold water the amount was 500.0 mg, suggesting that under these conditions L-theanine is not degraded by heat.

3.3. Brewing time

In Experiment 2, the brewing time curve shown in Fig. 1 shows that there was little difference in the amount of L-theanine extracted when the preparation was allowed to cool compared with when it was kept at a constant 80 °C. Any variations are likely to be due to the different movements and interactions of the tea bags inside the beakers during infusion. The amount of L-theanine increases rapidly in the first 5 min showing that infusion time during this period has a large effect on the amount of L-theanine released. The rate at which the L-theanine infuses is proportional

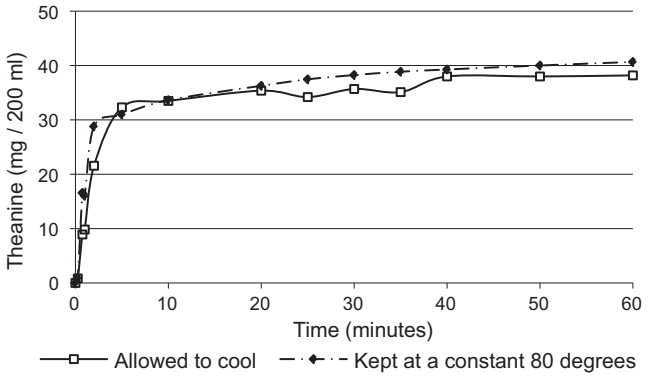

Fig. 1. Amount of L-theanine extracted as a function of brewing time. One sample was allowed to cool and the other was kept at a constant temperature.

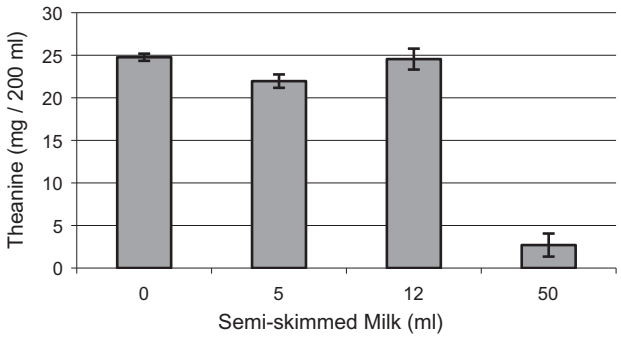

Fig. 2. Mean ± SE amounts of L-theanine in cups (200 ml) of tea with added amounts of semi-skimmed milk.

to the concentration of L-theanine in tea. Hence, as the concentration of L-theanine decreases in tea, the diffusion rate decreases. Astill et al. (2001) found a similar infusion curve over 3 min and Kyle et al. (2007) found that total phenols, catechins and antioxidants levelled off after the first 7 min, suggesting this effect is not confined to L-theanine. The large amount of L-theanine released in the first 5 min is also likely to be affected by the agitation method of adding the water to the tea bags, and the momentum of the interactions of the three tea bags inside the beaker. Amounts of L-theanine continued to increase to a small extent thereafter, showing that tea bags left in the cup or tea bags infused in teapots are likely to yield slightly more L-theanine. However, tea is probably usually consumed within the hour.

3.4. pH level

Experiment 3 investigated the effects of pH level. Before brewing, the pH of the de-ionised water was adjusted to pH 3, 7 or 9. These values changed to pH 4.3, 5.1 and 5.0 after the tea sample was added. The amount of L-theanine extracted from the pH adjusted tea samples was very similar (28.7, 27.9, 28.7 mg/200 ml respectively) suggesting that the pH level of the water is not having an effect on the amount of L-theanine extracted.

3.5. Addition of sugar

The addition of sugar (Experiment 4) resulted in 23.8 ± 1.53 mg L-theanine/200 ml compared to 23.9 ± 0.75 mg L-theanine/200 ml and made no significant difference to the amount of L-theanine extracted. Only one teaspoon of sugar was added (4.2 g); addition of larger amounts of sugar was not investigated.

3.6. Addition of milk

Experiment 5 investigated the effects of the addition of milk on the amounts of L-theanine extracted. The fat content of milk had only a small effect on the amount of L-theanine extracted from tea prepared in the standard manner. Amounts of L-theanine extracted are as follows: no milk (24.8 ± 0.73 mg/200 ml), skimmed milk (26.8 ± 1.76 mg/200 ml), semi-skimmed milk (24.5 ± 2.13 mg/200 ml) and full-fat milk (27.1 ± 3.31 mg/200 ml). Statistical analysis revealed no significant differences between these amounts. The volume of milk added, however, did affect the amount of L-theanine extracted ($p < 0.0001$). Fig. 2 shows that addition of 50 ml semi-skimmed milk greatly reduced the amount of L-theanine extracted ($p < 0.0001$, Tukey *post hoc* test). From examination of the chromatograms in Fig. 3 it appears that addition of 50 ml milk caused the appearance of a less polar peak. It is suggested that the amount of free L-theanine has decreased due to a binding process. It is unknown whether the L-theanine would be irreversibly bound or would be released again in the gut and still be absorbed by the body. Further analysis is required to determine the amount of L-theanine available for absorption when tea is prepared with a large amount of milk. The recommended standard amount of milk added to a tea preparation is 12 ml (Tetley Tea) and this made no significant difference to the amount of L-theanine extracted.

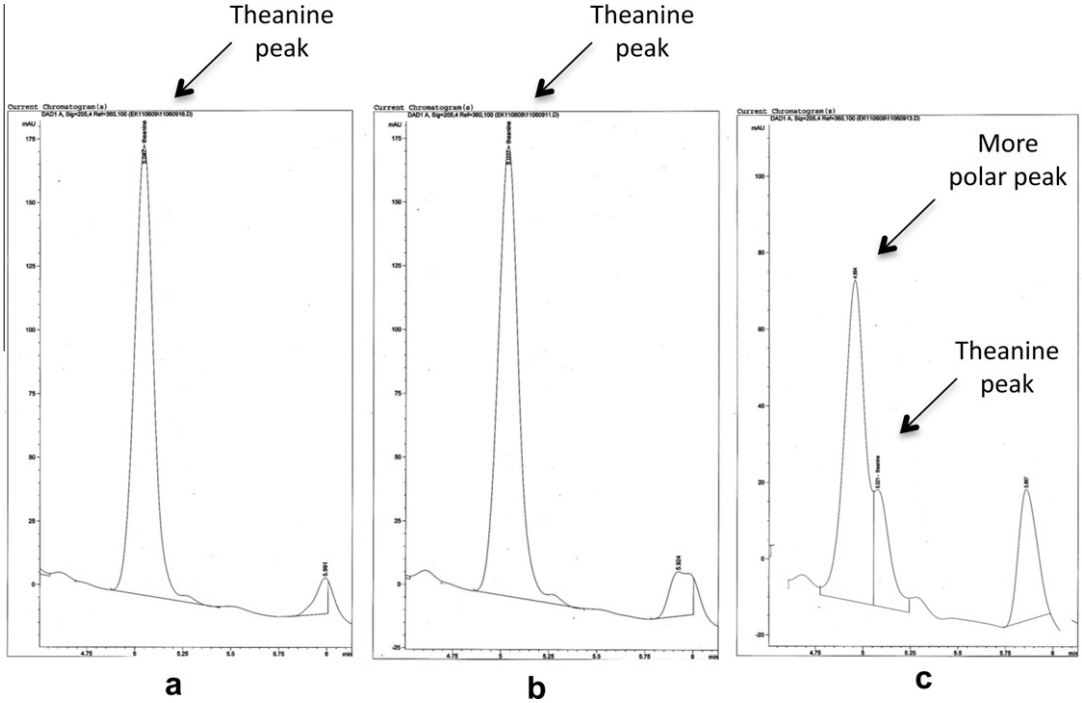

Fig. 3. Chromatograms of L-theanine peaks for (a) standard tea preparation (no milk), (b) tea with 12 ml semi-skimmed milk and (c) tea with 50 ml semi-skimmed milk.

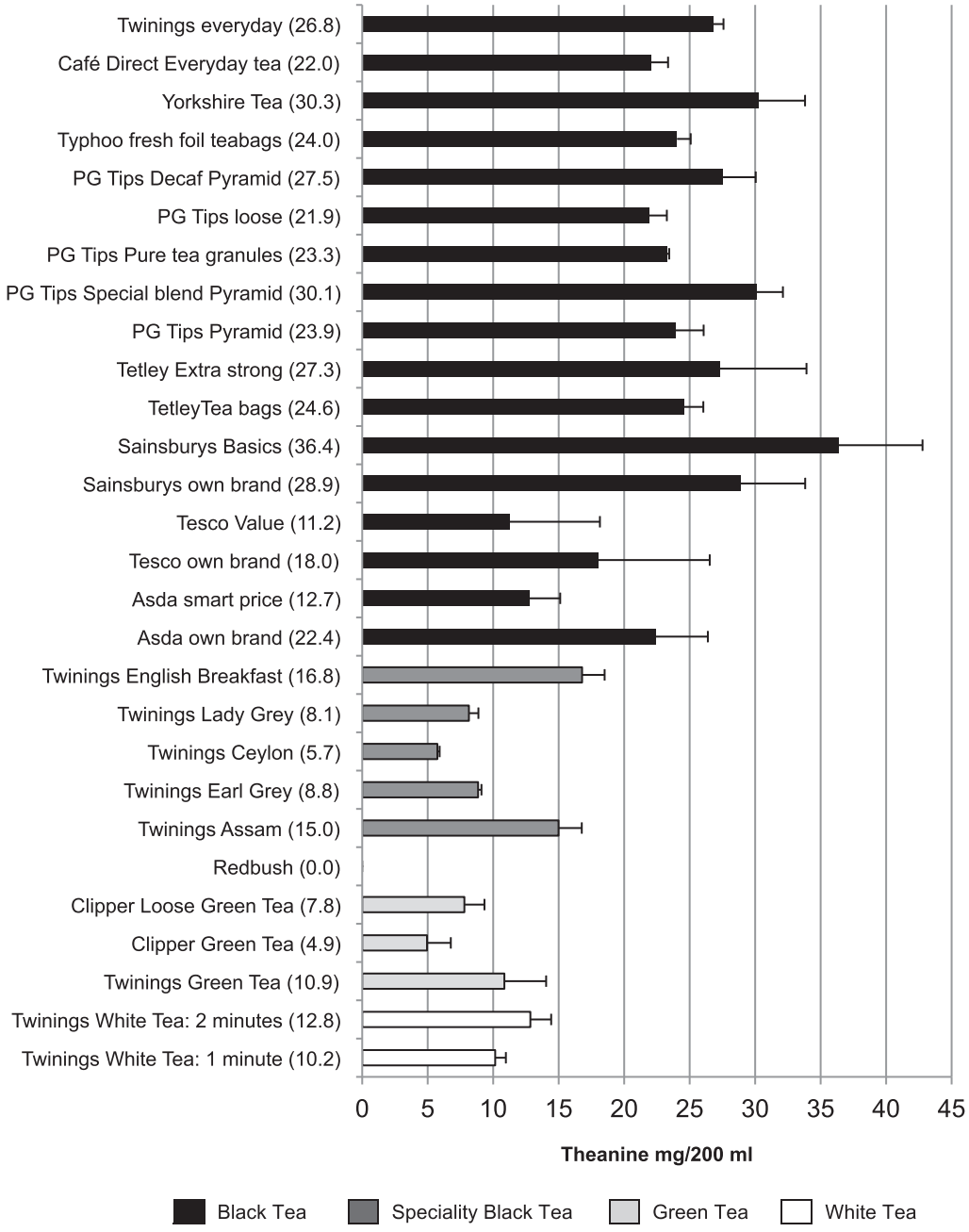

Fig. 4. Mean ± SE amounts of L-theanine in cups (200 ml) of commercially-available teas. Mean amounts of L-theanine (mg/200 ml) are also shown in brackets.

3.7. Effects of agitation method

Astill et al. (2001) showed the effects of agitation on the amounts of caffeine released. They suggested that agitation would be a variable with a significant contribution to the final concentration of the extract. Experiment 6 investigated the effects of agitation on the amounts of L-theanine extracted. Amounts of theanine extracted are as follows: water added to bag with squeeze upon removal (27.8 ± 0.86 mg/200 ml), water added to bag with no squeeze (24.3 ± 1.25 mg/200 ml), bag added to water with squeeze upon removal (24.8 ± 0.73 mg/200 ml) and bag added to water with no squeeze (22.4 ± 1.84 mg/200 ml). Comparison of the amount and type of agitation used shows the most vigorous method (water added to bag and tea bag squeezed upon removal) enhanced the extraction of L-theanine (5.39 mg/200 ml), compared to the least vigorous method. There were significant differences in the amount of L-theanine extracted dependent on whether water is added to the bag or the bag to water (2.47 mg/200 ml, $p < 0.01$, and whether the bag is squeezed upon removal or not (2.93 mg/200 ml, $p < 0.01$. These two factors were independent of one another (no interaction effect). It appears that the effects of agitation could be responsible for a variation of ± 5 mg/l L-theanine/200 ml cup.

There are additional factors that would be likely to affect the amount of L-theanine extracted from the tea preparations including the brand of tea, the size and shape of the tea bag and the amount of tea contained within each tea bag.

3.8. Various tea brands

Brand-dependent variations of L-theanine extraction were assessed in Experiment 7. The teas assessed here were all from

Camellia sinensis, apart from Redbush tea, which comes from *Aspalathus linearis*. Redbush tea is naturally caffeine free; it is also L-theanine free (Fig. 4). As it was not known whether the removal of caffeine from tea would also affect the amount of L-theanine present, PG Tips decaffeinated tea was extracted and found to contain significantly more L-theanine than standard PG Tips ($p = 0.005$, Fig. 4). This was the only brand of decaffeinated tea tested so further analysis is required to determine whether this is the same for all decaffeinated teas.

The Twinings speciality teas tended to contain less L-theanine than the other black teas (Fig. 4), which supports previous studies that black teas of higher quality yield less L-theanine (Feldheim et al., 1986; Ying et al., 2005). Unlike most of the other tea varieties tested, these teas were packaged in double-chamber bags. Some of the cheaper own-brand teas (Tesco Value and Asda Smart Price) also contained low L-theanine levels. These teas were particularly 'dusty', compared to others.

The relatively small amounts of L-theanine found in white and green tea as measured both by cup and standardised by gram was surprising, as previous studies have indicated that white and green teas should contain the most L-theanine, due to being unfermented (Alcazar et al., 2007; Hilal & Engelhardt, 2007; Ying et al., 2005). The explanation for these different results is not entirely clear, but probably involves several factors. Previous studies used much longer brewing times (Alcazar et al., 2007; Ying et al., 2005), although Fig. 5 shows how less L-theanine is extracted from green tea than black tea at all brewing times up to 30 min. There were also no significant differences in the size of leaf particles contained within the tea bags (large > 710 μm: 0.99 g *vs.* 0.74 g; medium 300–710 μm: 1.68 g *vs.* 1.62 g; small < 300 μm: 0.10 g *vs.* 0.33 g for PG Tips and Twinings green, respectively), which has been suggested to be an important determinant of the extraction efficiency (Hilal & Engelhardt, 2007). It is often recommended to use water that is 'off boiling' for brewing green and white tea. Water of around 80 °C was used here, and it was also found that hot water did not reduce the amount of L-theanine extracted. Various HPLC methods have been used by other workers with different mobile phases, column temperatures, fluorescence detection parameters and flow rates. The variation in retention time found in this study was a probable effect of small variations in the volatile component of the mobile phase. Within a run the retention time was consistent with that of the standards. Separation was always achieved in less than 10 min. Hilal and Engelhardt (2007) found that white tea contained the most L-theanine and used a polyamide column to remove polyphenols. Ying et al. (2005) did not assess white tea but found that green tea contained more L-theanine than darker teas. Their extraction method included boiling their samples for 10 min, which is very different from the method employed here, and may explain the differences to our results. The tea samples used in all these studies (including our own) are from various parts of the world, and as Hilal and Engelhardt (2007) noted, there is no agreed definition of white tea. Hilal and Engelhardt (2007) purchased various tea samples from the German market, Ye Ying et al. (2005) from various provinces in China, Alcazar et al. (2007) purchased commercial tea samples from local retail shops in Spain, and our own were commercial samples from UK shops. However, the Twinings White Tea used here is a combination of both definitions of white tea, where the unopened buds and top leaves of tea plants from the Fujian province of China are withered and dried only (Twinings White Tea). This makes it unlikely that the different results can be explained by the location of tea production. Even though green and white tea have been found to contain the most L-theanine due to being unfermented (Alcazar et al., 2007; Hilal & Engelhardt, 2007; Ying et al., 2005), green tea has been found to contain the most polyphenols (Scalbert & Williamson, 2000) and could suggest that the L-theanine has been converted to polyphenols (Kyle et al., 2007) by sunlight. However, this would not apply to white tea, as it is often shielded from sunlight to reduce the formation of chlorophyll to give a white appearance (Alcazar et al., 2007). The amount of L-theanine found in green tea in the present study ranged from 2.58–4.23 mg/g and has ranged from 1.31–4.16 mg/g in previous studies (Alcazar et al., 2007; Syu et al., 2008; Thippeswamy et al., 2006). The amount of L-theanine determined in tea made from PG Tips loose tea (21.9 mg/200 ml) was almost identical to the amount stated on the packet (22 mg/200 ml). This lends confidence in the method used here. Table 2 summarises data by tea type. Statistical analysis revealed a significant overall difference in amounts of L-theanine between tea types ($p < 0.001$), with *post hoc* tests revealing differences between black tea and all other types of tea (green tea and black speciality tea both $p < .001$; white tea $p < 0.05$). Differences between green, white and speciality black teas were not significant.

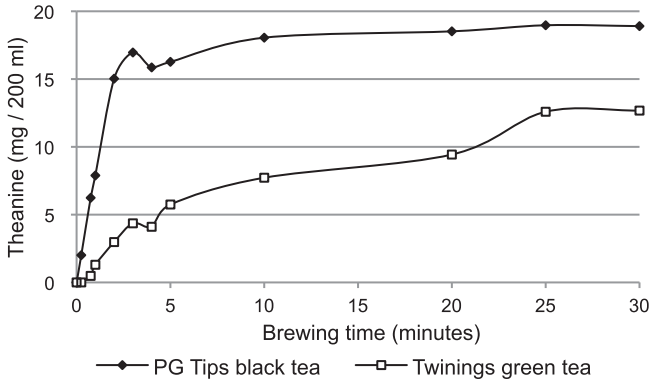

Fig. 5. Amount of L-theanine as a function of brewing time for one black tea (PG Tips pyramid bags) and one green tea (Twinings green tea bags). Results have been standardised so that preparations consist of equal weights of tea (6 g each).

Table 2
Amounts of L-theanine by gram of tea and by standard cup in popular commercial tea types at 2 min brewing time.

	Theanine/gram	Theanine/standard cup
White tea (2)[a]	4.5	11.5 ± 2.1[b]
Green tea (3)	3.6	7.9 ± 3.8
Black speciality tea (5)	5.3	10.9 ± 1.6
Black tea (17)	9.1	24.2 ± 5.7

[a] Name (N).
[b] Value (mg/200 ml) = mean ± standard deviation.

4. Conclusion

The amount of theanine present in black tea samples was significantly greater than in either black speciality, green or white tea varieties. The addition of moderate amounts of milk and sugar do not, on the other hand, appear to affect the amount of L-theanine extracted, and therefore can be ignored when estimating L-theanine consumption. These data provide a valuable starting point for converting information on frequency of tea consumption to amounts of L-theanine consumed. For a more accurate assessment it is recommended that the brewing time also be determined, as there are large variations in the amount of L-theanine released during the first 5 min of brewing. Brand of tea and amount of agitation during preparation are also important but, given the wide variety of teas available to consumers and the subtlety of differences in brewing behaviour (apart from brewing time), more difficult to account for. Caution should be exercised in future reports of L-theanine intake if based on undefined sources of tea and its subsequent preparation.

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