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The content of bioactive constituents as a quality index for Vietnamese teas

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Abstract: Although Vietnam is one of the largest tea producing countries in the world, there is only limited data on the quality of Vietnamese grown teas. To provide a general measure of quality for Vietnamese grown teas, the content of catechins and caffeine in Vietnamese green, black and oolong teas was determined and compared with teas from other countries. The results showed that the content of catechins and caffeine in Vietnamese grown green teas was 70 mg/g and 21 mg/g whereas the levels were 34 mg/g and 19 mg/g in Vietnamese oolong teas and 12 mg/g and 20 mg/g in Vietnamese black teas, respectively. The findings also indicated that, despite some minor differences, the three types of Vietnamese teas were found to be comparable to their Chinese, Japanese and Australian marketplace counterparts.

Keywords: Vietnamese tea, green tea, black tea, oolong tea, catechins, caffeine

Introduction

Tea (*Camellia sinensis*) is native to the southern provinces of China and parts of India, Laos, Thailand, Vietnam, and Myanmar (Balentine et al., 1998). However, tea is presently cultivated in over thirty countries around the world, and the tea beverage is second only to water in terms of worldwide consumption (Graham, 1992; Wong et al., 2009). Based on the fermentation and oxidation of the polyphenols in the tea leaves during production, tea has been classified into three types; green tea, black tea and oolong tea (Hara, 2001). Green tea is referred to as non-fermented tea, in which the oxidation of the tea polyphenols called catechins is prevented and thus, most of the catechins are preserved during its processing. Black tea refers to fully fermented tea and oolong tea is semi-fermented tea. In these teas, aerobic oxidation of the tea leaf polyphenolics is allowed to occurr and the catechins are enzymatically catalysed to form theaflavins and thearubigins (Graham, 1992; Wan et al., 2009). For black tea the reaction is promoted to maximise the oxidation (fermentation) but for oolong tea it is stopped usually half-way before it is complete.

There are two main types of black tea, CTC (crushing, tearing and curling) black tea and orthodox (rolling) black tea, produced through various stages including withering, rolling, drying and grading (Astil et al., 2001). Unlike green and oolong teas which are not graded at the final stage of processing, black tea is graded based on the particle sizes to form various grades such as orange pekoe (OP), pekoe (P), broken orange pekoe (BOP), broken orange pekoe fanning (BOPF), fanning (F) and dust (Balentine et al., 1998).

The composition of tea varies with the climate, season, agricultural practices, varieties of plant, age of leaf, types of leaf and processing methods (Balentine et al., 1998; Lin et al., 2003; Yao et al., 2009). The catechin content is up to 30% of the dry weight, whereas, the content of caffeine is up to 5% of the dry weight (Graham, 1992; Chu and Juneja, 1997). There are four major catechins (Figure 1); in order of abundance, they are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) (Chu and Juneja, 1997).

Numerous human, animal, and *in vitro* studies have shown that the tea catechins are related to the prevention of certain types of cancers, a reduction in the risk of cardiovascular diseases, an improvement of the immune system and the prevention of obesity and diabetes (Dufresne and Farnworth, 2001; Koketsu, 1997; Sajilata et al., 2008; Sharangi, 2009; Wheeler and Wheeler, 2004). Studies have also found that caffeine can act as a diuretic, a cardiac stimulant, a central nervous system stimulant and a smooth muscle

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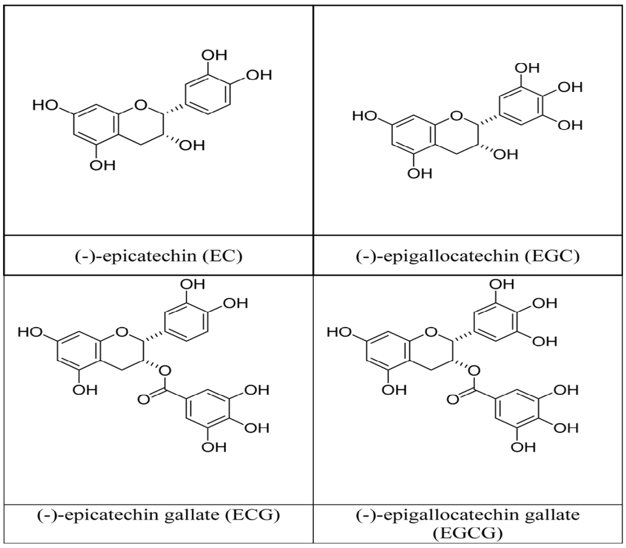

Figure 1. Chemical structure of the major catechins.

relaxant. However, caffeine may cause irritation of the gastrointestinal tract and sleeplessness (Chu and Juneja, 1997). The catechins and caffeine are also important for the quality of teas because they contribute to the astringency and bitterness of tea infusions (Jiang, 2009).

In Vietnam, tea is mostly grown in the northern highlands at latitudes over 600 meters above sea level and in the midland areas in the middle of the country at 300 to 500 meters above sea level (Zeiss and Braber, 2001). Thai Nguyen and Lam Dong provinces (Figure 2) are representative of these two major tea producing regions in Vietnam. The country has a long history of growing and drinking tea; tea production was industrialised in 1918 when the French occupied Indochina and established tea plantations and tea factories in the area (Zeiss and Braber, 2001). Vietnamese tea production has significantly increased over the last 10 years; production increased from 43 000 tonnes in 1996 to 164 000 tons in 2007 (FAO, 2009). Similarly, tea exports has also increased dramatically from 57 000 tonnes in 2003 to 106 000 tonnes in 2006 (FAO, 2008).

In 2006, Vietnam was ranked seventh in the world for tea production with 133 000 tons, in which green tea accounted for 66 000 tons and black tea 67 000 tons (FAO, 2008). Despite its significance to world production, there is limited data on Vietnamese tea quality. Therefore, in this study, the major chemical bioactive components of teas, the catechins and caffeine, were determined in Vietnamese green, black and oolong teas. In addition, Japanese green tea, Chinese green and oolong teas, and some black teas commercially available in Australia were analysed for comparison with the Vietnamese teas.

Materials and Methods

Materials

To quantify the quality of various Vietnamese grown teas, eight green tea samples, three oolong tea samples and seven types of orthodox black teas were purchased from Thai Nguyen and Lam Dong provinces. For comparison, fifteen Japanese green tea samples, eight Chinese green tea samples, three Chinese oolong tea samples and six black tea samples were purchased from the Australian marketplace for analysis.

Chemicals

The following chemicals were used for analysis: L-tryptophan (used as an internal standard), acetonitrile, ortho-phosphoric acid, teterahydrofuran, caffeine, and the catechins (EC, EGC, EGCG, and ECG) were all obtained from Sigma Chem. Co. (Castle Hill, NSW, Australia).

Preparation of tea infusions

One gram of each tea sample was brewed in 100ml of boiling de-ionized water for 20 minutes. The infusions were then immediately put on ice to cool down to 15°C, filtered through 0.45µm Alltech cellulose syringe filters and transferred to brown vials (Alltech, Baulkham Hills, NSW, Australia). The

Figure 2. The geographical location of the two major tea growing regions in Vietnam. Thai Nguyen is in the north of the country and is close to the capital Hanoi whereas Lam Dong is in the midlands and is close to Ho Chi Minh City.

infusions were then injected onto a high performance liquid chromatography (HPLC) system for analysis.

Analytical determination

Analysis was conducted using a Shimadzu HPLC system (Rydalmere, NSW, Australia). The chromatographic separation was performed on a 4µm x 250 x 4.6 mm C18 reversed-phase column (Phenomenex, Lane Cove, NSW, Australia) maintained at 25°C. The spectra were recorded at 210 and 280 nm using a UV-Vis detector. The mobile phase consisted of solvent A: 0.2% (v/v) orthophosphoric acid/acetonitrile/tetrahydrofuran, 95.5/3/1.5% (v/v/v) and solvent B: 0.2% (v/v) orthophosphoric acid/ acetonitrile /tetrahydrofuran, 73.5/25/1.5 (v/v/v). A gradient elution schedule was used: 0-10 min, 100% A; 10-40 min, linear gradient from 0 to 100% B; 40-50 min, linear gradient from 100 to 0% B with a post-run re-equilibration time of 10 minutes with 100% A before the next injection. The flow rate was 1 ml/min. An auto injector was used to inject 20 µl of the tea infusions onto the HPLC column. The quantification of the tea components was done by comparing the ratios of the peak areas of each tea component to the peak area of the internal standard L-tryptophan, to standard curves for each of the pure component external standards. A representative chromatogram is shown in Figure 3.

Data analysis

Linear regression analysis of the curves was done using Excel 6.0. One-way ANOVA analysis and the Fisher Least Significant Difference (LSD) post hoc test were done using the SPSS statistical software version 12.0 for Windows. Differences in the mean levels of the components in the different tea samples were taken to be statistically significant at $P<0.05$.

Results and Discussions

Composition of green teas produced from Thai Nguyen and Lam Dong provinces

The composition of the green teas produced in Thai Nguyen and Lam Dong provinces, which are representative of the northern highland and middle highland regions of Vietnam, respectively, are shown in Table 1. The results show that green teas produced from the two provinces had similar levels of caffeine, total catechins, EGCG and EGC. However, the green teas produced in Thai Nguyen province had significantly lower levels of ECG and EC compared to green teas produced in Lam Dong province ($P<0.05$). The variation in the levels of the individual catechins may have been influenced by the different cultivation conditions in the two regions or to seasonal differences. The northern highland region of Vietnam is relatively warm during the day and cool at night whereas the midland region is cooler but more humid. The rainfall is also higher in the midland region, especially during the summer months (Heiss and Heiss, 2007). However, the seasonal and environmental conditions under which the different teas were grown and harvested were not known.

Composition of Vietnamese orthodox black teas

The levels of total catechins and caffeine in the seven orthodox black tea grades were analysed to obtain a general quality index for Vietnamese black tea. These black tea grades comprised of pekoe (P), pekoe broken super (PBS), orange pekoe super (OPS), orange pekoe A (OPA), orange pekoe (OP), orange pekoe fanning (OPF) and pekoe super (PS). The results are shown in Table 2. The P and PBS black tea grades had the highest content of total catechins, followed by the OPF, OP and PS black tea grades, whilst the OP super and OPA black tea grades had the lowest content of total catechins. The content of caffeine also differed between the various grades of black tea. The P black tea grade had the highest content whereas the OPA black tea grade had the lowest content of caffeine. The variation in content of catechins and caffeine between the different grades can be explained by either the influence of the duration and temperature during fermentation, which results in various degrees of oxidative degradation of catechins (Cloughley, 1983), or to differences in the chemical composition in the different parts of the leaf, which are broken into different sizes after rolling to obtain the different grades (Lin et al., 1996; Balentine et al., 1998).

Comparison of Vietnamese green, black and oolong teas

The composition of Vietnamese green, black and oolong teas is shown in Figure 4. The results show that Vietnamese green teas had a significantly higher content of total catechins (70 mg/g) as compared to black (12 mg/g) or oolong teas (34 mg/g) ($P<0.05$). These results are in agreement with findings from other studies, which have shown that green tea contains the highest amount of catechins, followed by oolong tea, and black tea has the lowest content of catechins (Cabrera et al., 2003; Lin et al., 2003; Peterson et al., 2005)

The variation in total catechin content can be explained by the activities of the enzymes polyphenol oxidase (PPO) and peroxidase (POD), which are responsible for the oxidative conversion of catechins

Table 1. The composition of green teas from two representative Vietnamese provinces

	Thai Nguyen (n = 4)	**Lam Dong** (n = 4)
Caffeine (mg/g)	20.6 ± 0.8	22.2 ± 0.9
Total catechins (mg/g)	72.4 ± 5.2	76.1 ± 3.4
EGCG (mg/g)	40.1 ± 3.6	40.1 ± 1.0
EGC (mg/g)	13.1 ± 0.5	12.4 ± 1.2
EC (mg/g)	10.8 ± 0.6	13.0* ± 1.4
ECG (mg/g)	8.3 ± 0.8	10.5* ± 0.4

The letter 'n' refers to the number of different teas. The analysis of each tea was repeated six times and the values are expressed as mean ± SE in mg of constituent per gram of dry tea (mg/g). Values with the asterix '*' symbol are significantly different ($P < 0.05$) from the other value in the same row.

Table 2. The composition of Vietnamese orthodox black teas

Black tea grades	**n**	**Caffeine** (mg/g)	**Total catechins** (mg/g)
P	4	$24.9^{a} \pm 1.6$	$12.9^{a} \pm 1.0$
PBS	4	$20.4^{b} \pm 2.7$	$12.2^{a} \pm 0.9$
OP super	4	$18.6^{bc} \pm 1.2$	$8.9^{b} \pm 0.3$
OPA	4	$14.1^{d} \pm 4.4$	$9.0^{b} \pm 0.3$
OP	4	$17.5^{bcd} \pm 0.5$	$10.9^{c} \pm 0.7$
OPF	4	$22.4^{ab} \pm 0.4$	$16.1^{d} \pm 0.7$
PS	4	$18.0^{bc} \pm 0.15$	$10.9^{c} \pm 0.2$

The letter 'n' refers to the number of different teas. The analysis of each tea was repeated six times and the values are expressed as mean ± SE in mg of constituent per gram of dry tea (mg/g). Values in the same column not sharing the same superscript letters are significantly different ($P < 0.05$) from each other. The abbreviations used for the black teas grades are P: pekoe; PBS: pekoe broken super; OPS: orange pekoe super; OPA: orange pekoe A; OP: orange pekoe; OPF: orange pekoe fanning; and PS: pekoe super.

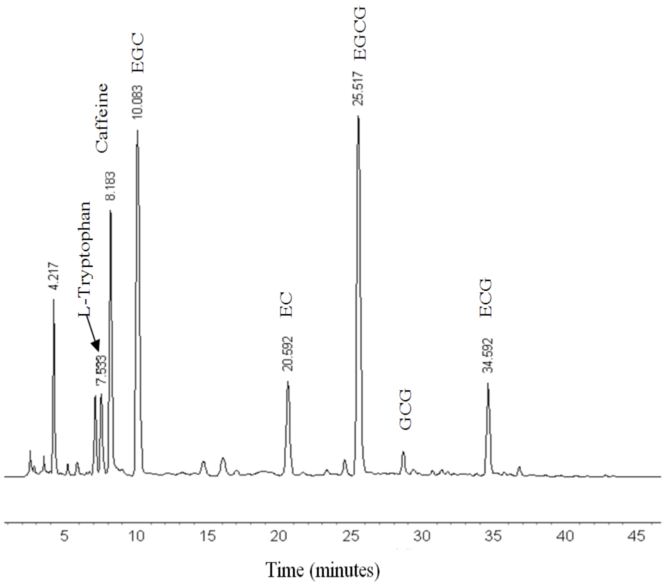

Figure 3. A representative HPLC chromatogram of a green tea sample run on a Shimadzu HPLC system with UV detection at 210 nm.

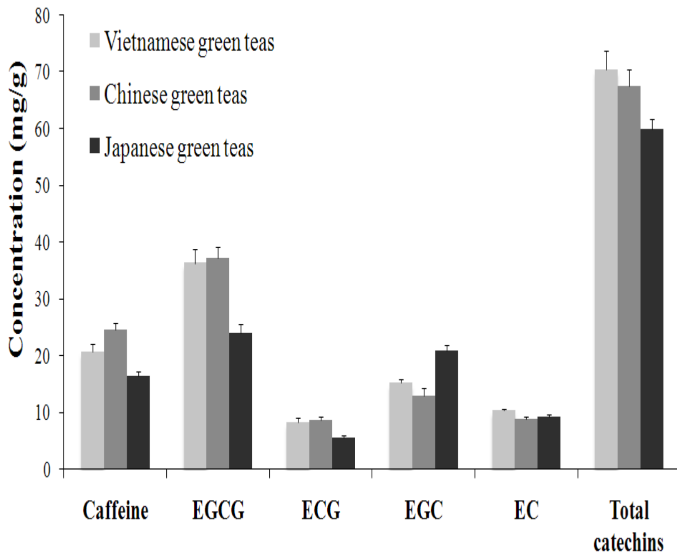

Figure 5. Comparison of the composition of eight Vietnamese green teas with eight Chinese and 15 Japanese green teas. The analysis of each tea was repeated six times and the values are expressed as mean + SE in mg of constituent per gram of dry tea (mg/g).

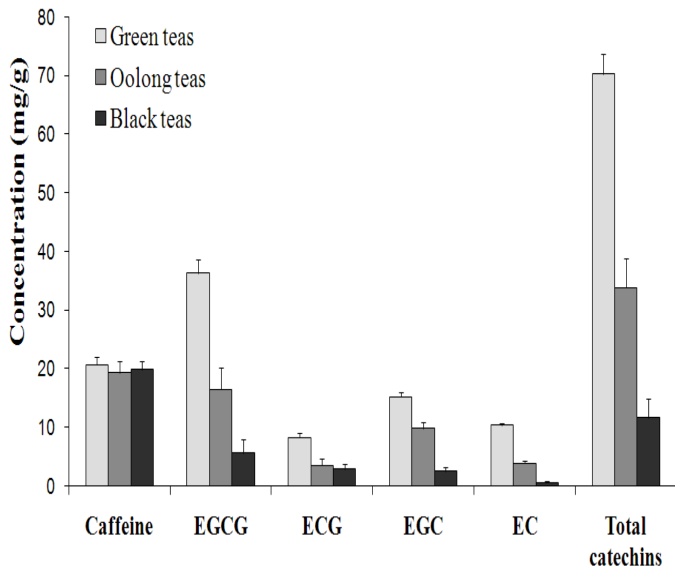

Figure 4. Comparison of the composition of eight green teas, three oolong teas and 28 black teas from Vietnam. The analysis of each tea was repeated six times and the values are expressed as mean + SE in mg of constituent per gram of dry tea (mg/g).

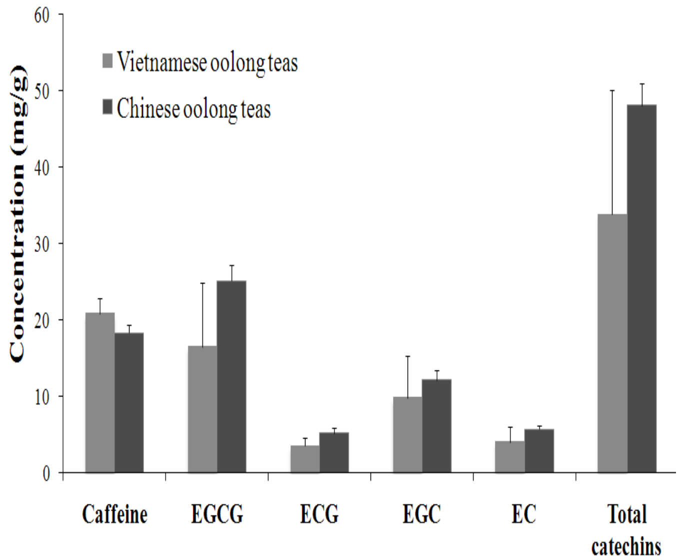

Figure 6. Comparison of the composition of three Vietnamese oolong teas with three Chinese oolong teas. The analysis of each tea was repeated six times and the values are expressed as mean + SE in mg of constituent per gram of dry tea (mg/g).

to theaflavins and thearubigins (Graham, 1992). The PPO and POD enzymes are inactivated early by heat in the green tea manufacturing process and thus the degradation of catechins is minimised. However, these enzymes are not inactivated and left to almost completely oxidize the catechins in black tea but to only partially oxidise them during the making of oolong tea. Consequently black teas have much less catechins than oolong and green teas (Graham, 1992).

The content of the four major catechins, EGCG, EGC, ECG and EC in the Vietnamese green teas (36, 8, 15 and 10 mg/g, respectively) were significantly higher than in the Vietnamese oolong (17, 4, 10 and 4 mg/g, respectively) and black teas (6, 3, 3 and 1 mg/g, respectively) ($P<0.05$). However, the levels of EGC, ECG and EC were not significantly different between oolong and black tea samples. These results are in agreement with previous findings reported by Zuo et al. (2002) and Caberera et al. (2003), which showed that green teas contained much higher amounts of EGCG, EGC, ECG and EC than oolong or black teas.

Vietnamese green tea contained 21 mg/g caffeine, which was similar to that measured in oolong tea (19 mg/g) and black tea (20 mg/g). This finding is contrary to previous results. Zuo et al. (2002) reported that the caffeine level in oolong tea was much lower than in black or green teas. However, Yen and Chen (1996) reported that the level of caffeine in oolong tea was higher than in green and black teas. The contradiction can be explained by several factors such as season, varieties and agricultural practices which are known to influence the content of caffeine (Balentine et al., 1998; Yao et al., 2009). However, the seasonal and environmental conditions under which the different teas were grown and harvested were not known.

Comparison of Vietnamese green teas with Japanese and Chinese green teas

The composition of Vietnamese, Chinese and Japanese green teas is presented in Figure 5. The Vietnamese green teas had similar levels of total catechins and of the four major catechins compared to the Chinese green teas but had significantly higher levels than the Japanese green teas ($P<0.05$). These results are in agreement with previous findings reported by Saijo and Takeda (1999), which revealed that Chinese and Vietnamese green teas had a significantly higher content of total catechins compared to Japanese green teas. The level of EGCG in Chinese and Vietnamese green teas was also significantly higher than in the Japanese green teas.

The present study also found that the content of EC was similar between the Vietnamese and Japanese green teas but it was significantly higher than in the Chinese green teas. This is supported by previous results reported by Lin et al. (1998), in which they showed that the levels of EGC and EC were higher in Japanese green teas than in Chinese green teas.

This present study also found that the content of caffeine in the Vietnamese green teas was significantly lower than in the Chinese green teas but higher than in the Japanese green tea samples. These results are supported by previous findings reported by Takeda (1994) and Katsuyuki et al. (2006). Takeda (1994) found that the level of caffeine in Japanese teas was lower than in Chinese teas, whilst Katsuyuki et al. (2006) found that fresh Vietnamese green tea leaf (wet, not dried) had a relatively high content of caffeine.

Comparison of Vietnamese oolong teas with Chinese oolong teas

The current study also compared the quality of Vietnamese oolong teas to Chinese oolong teas. The results are presented in Figure 6. The levels of total catechins and of the four major catechins in the Vietnamese oolong teas were significantly lower than those in the Chinese oolong tea samples ($P<0.05$). However, the Vietnamese oolong teas were found to have a similar content of caffeine compared to the Chinese oolong teas. The variation in catechin levels between oolong teas is most likely to be explained by

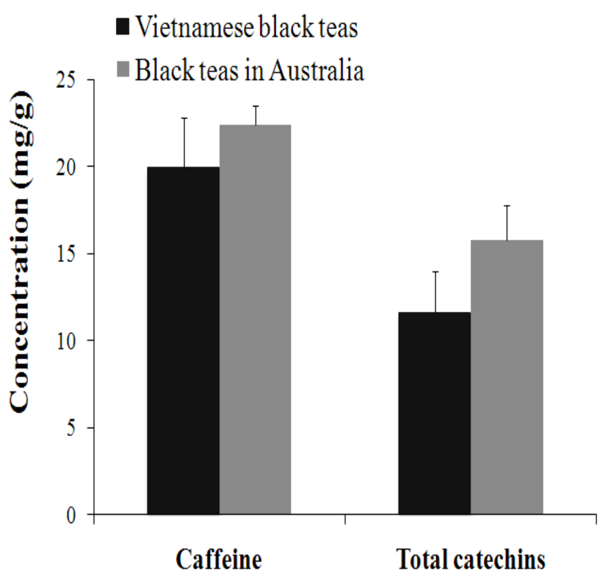

Figure 7. Comparison of the composition of 28 Vietnamese black teas with six black teas available on the Australian marketplace. The analysis of each tea was repeated six times and the values are expressed as mean + SE in mg of constituent per gram of dry tea (mg/g).

differences in the duration of the fermentation process, which partially converts catechins into theaflavins and thearubigins during the manufacture of oolong tea (Graham, 1992). In addition, other factors such as different varieties and agricultural practices could contribute to variation in the levels of catechins in oolong teas (Yao et al., 2009).

Comparison of Vietnamese black teas with black teas available on the Australian marketplace

The present study also compared the quality of Vietnamese orthodox black teas to some black teas available in Australia. The results showed that the levels of total catechins and caffeine in the Vietnamese black teas were significantly lower than those in the other black teas available in Australia (Figure 7). As for oolong teas, the variation in catechin content may be explained by differences in the duration of the fermentation step, which can easily result in different levels of oxidation of the catechins to theaflavins and thearubigins during black tea manufacture; the longer the time of fermentation, the lower the levels of total catechins are when the process is stopped (Hara, 2001; Graham, 1992). Similarly, Cloughley (1983) found that the level of caffeine was also influenced by the duration of the fermentation; the longer fermentation times resulted in lower amounts of caffeine. However, it should be noted that the quality of black teas is also contributed to by the theaflavins and thearubigins (Balentine et al., 1998). Thus, future studies are needed to determine the levels of theaflavins and thearubigins in Vietnamese black teas.

Conclusion

In this study, Vietnamese green tea samples from both Thai Nguyen and Lam Dong provinces were found to have high levels of total catechins and the four major catechins. The levels of these constituents in oolong tea samples were found to be lower than those in green teas and the black teas were found to have the lowest content of these components. In comparison with teas produced in other countries, Vietnamese green teas had similar levels to Chinese green teas but higher levels of catechins compared to the Japanese green teas. Vietnamese green teas had a lower content of caffeine compared to Chinese green teas but higher caffeine levels than Japanese green teas. Vietnamese oolong teas had a lower amount of catechins but a similar level of caffeine compared to Chinese oolong teas. Vietnamese black tea samples had a lower content of catechins and caffeine than the other black teas available in Australia. Despite some differences based on their bioactive constituents, the three types of Vietnamese teas were found to be fairly comparable to their Chinese, Japanese and Australian marketplace counterparts.

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