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Compositional analysis of teas from Australian supermarkets

Lihu Yao [a,c], Xu Liu [b], Yueming Jiang [a,*], Nola Caffin [c], Bruce D'Arcy [c], Riantong Singanusong [d], Nivedita Datta [c], Ying Xu [e]

[a] South China Botanic Garden, The Chinese Academy of Sciences, Guangzhou ReYiJu 510650, PR China
[b] Tea Research Institute, The Chinese Academy of Agricultural Sciences, Hangzhou 310008, PR China
[c] School of Land and Food Sciences, The University of Queensland, Brisbane, Queensland 4343, Australia
[d] Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environmental Sciences, Naresuan University, Muang, Phitsanulok 65000, Thailand
[e] College of Food Science, Southwest Agricultural University, Chongqing 400716, PR China

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Abstract

Caffeine, total amino acids, water extract and moisture content are considered to be quality indicators for leaf teas and teabags. These analyses were examined in 20 leaf teas and 36 teabags sampled from Australian supermarkets. About 70% of the analysed samples showed a moisture content higher than the maximum accepted level, 6.5%, for tea storage and marketing by the tea industries and traders. Water appropriate extract of 15 samples out of 36 teabags was lower than that of the teas without teabags, which indicates that the quality of the paper used for teabags needs to be evaluated. Moreover, one of the black leaf tea samples was found to have a water extract below the lower limit of international standards.

Four green and black teas of the same brand, claimed to contain less than 3% caffeine, were found to have 3–4%, the same as the other samples analysed in this study. The mean total contents of amino acids were 2.50% and 1.76% in black leaf teas and the teabags, respectively, whereas they were 3.44% and 2.28% in green leaf teas and the teabags, respectively. Furthermore, the weights of 28 teabags out of 36 samples were found to lie outside of the proposed ±2% variation accepted by the tea industries and traders, and 4 samples showed even larger variation, 10% being out of the proposed weights. This investigation also showed that the solubility of caffeine and water extract was affected by the permeability of teabags, whereas total amino acids were very variable.

These results suggest that an efficient and practical quality control system for both imported and Australian-made teas in the Australian supermarkets should be developed, implemented and enforced. Chemical analysis should be a part of the system for establishing an objective assessment for the quality control.

Keywords: Tea; Caffeine; Amino acids; Water extract; Spectrophotometric; Quality

1. Introduction

Tea is made from the young tender shoots (flushes) of *Camellia sinensis* (L.) O. Kuntze (Hara, Luo, Wickremasinghe, & Yamanishi, 1995; Wright, Mphangwe, Nyirenda, & Apostolides, 2002) and is the most widely consumed drink after water, due to its refreshing and mildly stimulant effects (Harbowy & Balentine, 1997). Alkaloids, caffeine (1,3,5-trimethylxanthine) and two minor isomeric dimethylxanthines, e.g., theobromine and theophyline, are responsible for mildly stimulant effects of tea (Cloughley, 1981; Stagg & Millin, 1975). Caffeine plays a vital role in tea quality contributions, such as briskness and other taste characteristics (Dev

* Corresponding author. Tel.: +86 20 37252905; fax: +86 20 37252831.
E-mail address: ymjiang@scib.ac.cn (Y. Jiang).

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Choudhury, Rahman, & Barbora, 1991; Hilton & Ellis, 1972; Roberts, 1962; Sanderson, 1972), and has been considered as an important quality parameter for the evaluation of tea quality (Khokhar & Magnusdottir, 2002; Owuor, Horita, Tsushida, & Murai, 1986).

Twenty-six amino acids have been identified in tea leaves, contributing to the taste of a brew and tea quality (Hara et al., 1995) and comprising about 3–4% of the dry matter of a tea (Millin & Rustige, 1967). Theanine is the principal (and a unique) amino acid found in tea and represents more than 50% of total amino acids of black tea (Hara et al., 1995; Harbowy & Balentine, 1997). Bhatia (1963) reported that the content of amino acids in tea could be as high as 7% of the dry matter, which can be infused completely into the brew. However, the infusion rate of amino acids from green tea is dependent on the type and clone of the tea (Harbowy & Balentine, 1997; Yao, Cheng, Chen, & Liu, 1992), and is not significantly affected by the infusion time after 3 min at infusion temperatures above 70 °C (Yao et al., 1992).

Water extract of a tea has long been regarded as an important international standard for quality control of tea (Hara et al., 1995; ISO 3720, 1986; ISO 9768, 1994). The water extract of tea consists of phenolic compounds, sugars, amino acids, alkaloids and many minor soluble substances, such as minerals and pigments (Harbowy & Balentine, 1997; Wood et al., 1964). Water extract has also been referred to as aqueous extraction, water-soluble extract and/or total solids of tea (Long, 1977; Millin & Swaine, 1981; Sanderson et al., 1976; Wood et al., 1964; Yao et al., 1992). The amount of water extract of tea is determined by a number of factors, e.g., tea/water ratio; temperature of the infusion; type, particle, size and constituents of a tea (Long, 1978, 1979; Yao et al., 1992; Yao, Chen, Cheng, & Liu, 1993).

The moisture content of a tea is paramount for the quality of tea during the storage period. Roberts and Smith (1963) found that an increase of moisture content is associated with quality loss of the tea. Othieno and Owuor (1984) reported that, if the moisture content of a tea is $\geqslant$6.5% or $\leqslant$2.5%, the quality of the tea is disastrous. This indicates that very high moisture contents are extremely detrimental to tea quality (Robinson & Owuor, 1993). In contrast, teas with moisture contents below 2.5% may have a smoky taint, which renders them equally unacceptable. Millin (1987) suggested that the desirable moisture content was about 3% for tea from the factory, because tea always absorbs moisture during transportation, storage and trading with a resultant moisture content of 7–8% before retail packing, which leads to substantial deterioration of tea quality.

Irrespective of packing method, moisture content of black tea increases gradually during the first three months of storage and then reaches a constant value (Obanda & Owuor, 1995). However, no studies have been reported on caffeine, amino acids a water extract of teas from Australian markets. Thus, the aim of this study was to quantify the tea constituents that might be used as indicators of the quality of teas in Australian and international markets.

2. Materials and methods

2.1. Samples

Leaf tea and teabags, commercially available from supermarkets in Queensland, Australia, were randomly sampled and used for this study, except for one leaf tea that was made in the laboratory from fresh tea leaf obtained from a commercial tea estate at Malanda in North Queensland. This leaf was used as a representative of freshly made Australian teas for the supermarkets for comparison in this study. One crude black tea sample, provided by the same manufacturer, was also used for comparison.

Teabags were either heat-sealed as in the UK-type or double chamber US-type machine. The sampling method used in this study was based on an international standard method (ISO 1839, 1980) as part of a study to survey the composition of teas from Australian supermarkets.

2.2. Moisture

Tea moisture was measured using a vacuum oven and the international standard method (ISO 1573, 1981).

2.3. Preparation of the tea solution

The preparation consisted of addition of boiling water (200 ml) to leaf tea (2 g) or teabag (1 bag equivalent to 2 g) in a 250 ml conical flask and stirred by a magnetic bar on a hot plate at 90 °C for 10 min. Then, the tea solution was filtered through cotton wool and the residue was washed with distilled water (3×10 ml). The tea solution cooled to room temperature and washings were diluted to 250 ml with distilled water. The sample was analysed in duplicate.

2.4. Caffeine, water extract and total amino acids

2.4.1. General methods

The methods used for the analysis of caffeine, water extract and amino acids of the tea solution were based on international standards (ISO 1839, 1980; ISO 9768, 1994; ISO 10727, 1995) with modification (Yao et al., 1992, Yao, Chen, et al., 1993) as detailed below.

2.4.2. Total amino acids

2.4.2.1. Buffer solution. Na_2HPO_4 (23.38 g) was dissolved in distilled water (1000 ml) and KH_2PO_4 (9.08 g) was dissolved in distilled water (1000 ml). A buffer solution consisting of 95% (v/v) of Na_2HPO_4 solution and 5% (v/v) of KH_2PO_4 solution, was prepared.

2.4.2.2. Ninhydrin solution. Ninhydrin (2 g) was dissolved in distilled water (50 ml) and $SnCl_2$ (80 mg) was added to the solution diluted to 100 ml with distilled water.

2.4.2.3. Measurement. Tea solution (1 ml), buffer solution (0.5 ml) and ninhydrin solution (0.5 ml) were placed in a 25 ml volumetric flask and the flask was heated in a boiling water bath for 15 min. Then, the flask cooled to room temperature and the solution in the flask was diluted to 25 ml with distilled water. The absorbance of the diluted solution was measured using a Pharmacia Ultrospec III UV/Visible spectrophotometer at 570 nm. This measurement was done in triplicate.

2.4.2.4. Standard curve. Theanine was used to prepare the standard curve. Theanine stock solution (1 mg/ml, w/v in distilled water; 5 ml) was diluted to 50 ml with distilled water. Then, 0, 1, 2, 3, 4, or 5 ml of the diluted theanine solution were separately added, each with buffer solution (0.5 ml) and ninhydrin solution (0.5 ml) to different 25 ml volumetric flasks. Each mixture was heated in a boiling water bath for 15 min. Then, the further dilution and measuring steps were repeated as described in the tea solution measurement. The readings of the absorbance of the standard solution against its concentrations were used to prepare the standard curve.

2.4.2.5. Calculation.

$$\text{Total amino acids } (\%) = E \times V_0 \times 100/1000/V_1/W = 0.1EV_0/V_1/W,$$

where E is mg of amino acids from the standard curve against the absorbance reading of the spectrophotometer; V_0 is the total volume of the tea solution (250 ml); V_1 is the volume used for the measurement (1.0 ml); W is the dry weight of the tea sample.

2.4.3. Caffeine

2.4.3.1. Lead acetate solution. $(CH_3COO)_2Pb$ (100 g) was dissolved and diluted to 200 ml with distilled water.

2.4.3.2. Hydrochloric acid solution. Hydrochloric acid (36% HCl, specific gravity 1.18, 0.9 ml) was diluted to 1000 ml with distilled water.

2.4.3.3. Sulfuric acid solution. Sulfuric acid (98% H_2SO_4, specific gravity 1.84, 167 ml) was diluted to 1000 ml with distilled water.

2.4.3.4. Measurement. Tea solution (10 ml), hydrochloric acid solution (5 ml) and lead acetate solution (1 ml) were mixed in a 100 ml volumetric flask and diluted to 100 ml with distilled water. The solution was then filtered through Whatman No. 1 qualitative filter paper. The filtrate (25 ml) and sulfuric acid solution (0.3 ml) were placed in a volumetric flask and diluted to 50 ml with distilled water. The solution was filtered using the same type of filter paper. The absorbance of the filtrate was measured using a Pharmacia Ultrospec III UV/Visible spectrophotometer at 274 nm. The measurement was performed in duplicate.

2.4.3.5. Standard curve. Caffeine stock solution (10 ml, 1 mg/ml, w/v in distilled water) was diluted to 200 ml with distilled water. Next, 0, 10, 20, 30, 40, or 50 ml of the diluted caffeine solution were separately mixed, each with hydrochloric acid solution (4 ml) in a volumetric flask and diluted to 100 ml with distilled water. Thereafter, the measuring steps were repeated as described in Section 2.4.3.4. The readings of the absorbance of the standard solution against its concentration were used to prepare the standard curve.

2.4.3.6. Calculation.

$$\text{Caffeine } (\%) = (E/1000) \times V_0 \times (100/V_1) \times (50/25)/W = 0.2EV_0/V_1/W,$$

where E is 'mg' of caffeine from the standard curve against the reading of the spectrophotometer, and E/1000 is to convert 'mg' into 'g'; V_0 is the total volume of the tea solution (250 ml); V_1 is the volume used for the measurement (10 ml), and $100/V_1$ indicates 10 ml tea solution that were diluted to 100 ml, while 50/25 shows that another dilution from 25 ml tea filtrate made to 50 ml in the measurement (Section 2.4.3.4); W is the dry weight of the tea sample.

2.4.4. Water extract

2.4.4.1. Measurement. Tea solution (50 ml) was placed in a weighed evaporation dish and was then evaporated to dryness over a water bath. The residue (tea extract) in the dish was fully dried in a vacuum oven at 75 °C with a negative pressure of 65 kPa for 4 h until the weight of the dish with extract was constant.

2.4.4.2. Calculation.

$$\text{Water extract } (\%) = (D_1 - D_0) \times V_0 \times 100/V_1/W,$$

where D_1 is the weight of dry tea extract with the dish; D_0 is the weight of the dish; V_0 is the total volume of the tea solution (250 ml); V_1 is the volume used for

the measurement (50 ml); W is the dry weight of the tea sample.

2.5. Solubility/permeability of caffeine, water extract and total amino acids in teabag

Determination of solubility/permeability of caffeine, water extract and total amino acids of the tea solution in teabags was performed with teabag and without teabag. The compositions were measured separately as described previously. The analyses were done in duplicate. The solubility/permeability values of these analyses were their contents measured with bags in relation to their contents measured without bags and expressed as a percentage.

All of the chemicals used in this study were of analytical grade. The percentage of each analysis was calculated on a dry weight basis (w/w).

3. Results and discussion

3.1. Caffeine

3.1.1. Black tea

The caffeine content of eighteen black leaf tea samples ranged from 3.31% to 4.61% (w/w), with a mean of 3.89% of the dry mass (Table 1). This was similar to the caffeine content in the 30 black teabags analysed, which ranged from 2.29% to 4.67%, with a mean of 3.87% (w/w). Earlier studies showed that caffeine content of black teas was affected by clone, season, and stage of plucking (Hara et al., 1995; Harbowy & Balentine, 1997; Owuor & Chavanji, 1986). The changing caffeine content due to the maturity between tea leaves can ranged from 24% to 40%, meaning that young leaves may contain more caffeine than older tea leaves. Owuor and Chavanji (1986) reported that caffeine content in one bud with five leaves had fallen to 2.91% (w/w) from one bud with two leaves of 4.88% (w/w). The caffeine content (Fig. 1) in the black teas from Australian supermarkets could be due to the tea clone, geographical locations, harvest time and infusion conditions (Hicks, Hsieh, & Bell, 1996; Owuor, 1992, 1994; Owuor, Obanda, Tsushida, Horita, & Murai, 1987; Yao et al., 1992). The results (Table 1) of the current study showed that the mean value of caffeine content in black leaf teas (3.89%) was close to that (3.87%) of the black teabags and the respective ranges were also very similar. Thus, the black teas from Australian markets have a generic coverage of teas from various clones, different production seasons and different maturities of harvest, according to the content of caffeine measured in these teas. The results may also indicate that the teas from Australian supermarkets are blended teas that may be from different sources with differences in clones, seasons, and maturity (see Fig. 1).

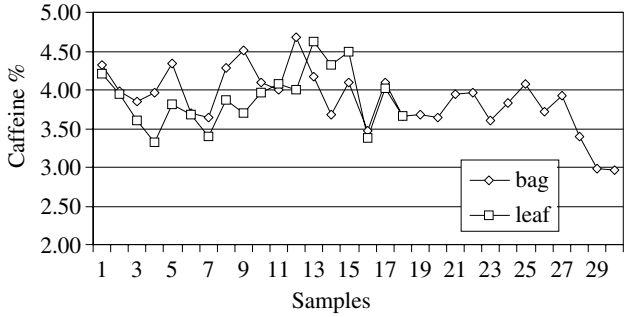

Fig. 1. Variation of caffeine content in black leaf teas and the teabags.

The caffeine contents in three Australian-grown and made black leaf teas showed an average of 3.36% (w/w), while they were 3.43% (w/w) of the dry mass in the five Australian-grown and made black teabags. Thus, the caffeine content in Australian made black teas (leaf tea and teabags) was lower than the mean content detected in the teas from the supermarkets (3.89% and 3.87%, respectively). The lower caffeine content of Australian grown teas could be due to late harvesting of more mature leaves than those of the imported teas (Owuor & Chavanji, 1986; Owuor et al., 1986).

3.1.2. Green tea

The caffeine content averaged at 3.83% in five green teabags, and 3.71% in two green leaf teas (Table 1), which are similar to the average value found in the black leaf teas and teabags, as discussed earlier.

Table 1
Contents of caffeine, amino acids and water extract in green and black teas

Type of tea	Caffeine				Amino acids				Water extract			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Black leaf (%) (n = 18)	3.31	4.61	3.89	0.37	1.71	3.63	2.50	0.53	31.50	39.85	36.20	2.22
Green leaf (%) (n = 2)	3.71	3.71	3.71	0.00	1.51	5.37	3.44	2.74	31.66	34.03	32.84	1.67
Black teabag (%) (n = 30)	2.29	4.67	3.87	0.39	1.11	2.68	1.76	0.43	32.01	41.96	37.39	2.46
Green teabag (%) (n = 6)[a]	3.47	4.29	3.83	0.42	1.73	3.06	2.28	0.58	36.02	42.50	39.18	2.22

[a] n = 5 for caffeine mean.

Table 2
Solubility or permeability of caffeine, amino acids and water extract in teabags

Teabags (n = 34)	Caffeine (%)				Amino acids (%)				Water extract (%)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Solubility or permeability	82.1	100	91.8	3.40	78.8	122	94.2	8.54	83.43	99.27	91.52	4.20

It is interesting to note that four Australian-grown produced black and green teabags were claimed to have a caffeine content below 3%. This study shows the claim to be unfounded. Nevertheless, there are no scientific data pointing to a preferable caffeine level in tea or tea products.

3.1.3. The solubility or permeability

The solubility or permeability of caffeine from teabags ranged from 82.1% to 100%, with a mean of 91.8% (Table 2). Although caffeine is a relatively stable component, the permeation of caffeine through teabags was found to be very variable. The reason for this variability is probably linked to the quality of the paper used for the manufacture of teabags. Therefore, the quality of paper used for teabags could usefully be reviewed for better quality control of tea.

3.2. Amino acids

3.2.1. Black tea

In the eighteen black leaf teas, total amino acids ranged from 1.71% to 3.63% (w/w), with a mean of 2.50% (w/w) (Table 1). These contents were much higher than the content detected in the 30 black teabags, which ranged from 1.11% to 2.68% with a mean of 1.76% (Table 1). These differences between leaf teas and teabags may be due to the infusion of amino acids being significantly affected by tea type and clone (Harbowy & Balentine, 1997; Yao et al., 1992, Yao, Chen, et al., 1993). Furthermore, the withering process during black tea processing may cause breakdown of protein to amino acids (Bhatia, 1964; Roberts & Wood, 1951), which may be another reason to explain the differences in amino acids between leaf teas and the corresponding teabags. It has been found that the total amino acids in fresh tea shoots comprising a bud and two adjoining leaves, can be as high as 7% of dry tea (Bhatia, 1963). Among these amino acids, theanine, a unique amino acid, represents 38–54% of total amino acids in green tea and more than 50% of total amino acids in black tea (Hara et al., 1995; Harbowy & Balentine, 1997). Generally, the content of amino acids is dependent on the tenderness of tea shoots, which is the same as the content of caffeine (Harbowy & Balentine, 1997). Moreover, the content of total amino acids is considered to be one of the parameters for tea quality assurance (Hara et al., 1995; Liang, Liu, Xu, & Hu, 1990; Yao et al., 1992). Thus, the amino acid content contributes to the total quality of a tea, being a contributor to the taste and colour.

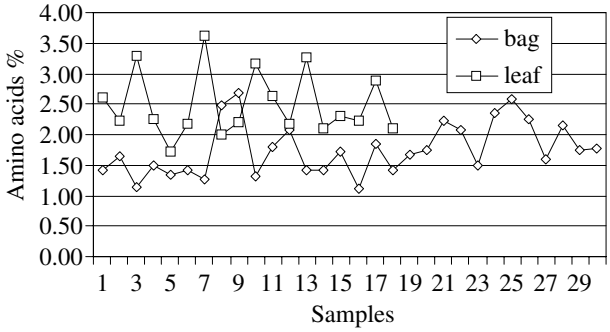

Fig. 2. Variation of content of amino acids in black leaf teas and the teabags.

The mean content of total amino acids in the three Australian-made black leaf teas analysed was 2.70%, while it was 2.10% in the five Australian-made black teabags (Fig. 2). Thus, the content of total amino acids in Australian made black teas (leaf tea and teabags) is, in general, slightly higher than the mean content of the teas from the supermarkets (2.50% and 1.76%, respectively). The difference in amino acid content highlights the variable nature of these compounds, as they are not only present as naturally-occurring compounds in the fresh shoots, but also present as breakdown products of protein during the manufacture (Bhatia, 1964; Liang et al., 1990; Owuor, Wanyiera, Njeru, Munavu, & Bhatt, 1989). Neither the dramatic change of amino acid content during processing and storage nor the origin of the amino acids is fully understood (Hara et al., 1995; Harbowy & Balentine, 1997).

Comparison with the amino acid content from previous studies showed that the amount in the black leaf teas in this study, at 2.50%, was greater than some (2%) reported, but less than others (3–4%) (Bhatia, 1963; Harbowy & Balentine, 1997; Millin & Rustige, 1967). However, even the mean amino acid content for black teabags from Australia markets showed a lower value (1.76%), which surely indicates a lower quality for teabags than for leaf teas. There may also be some absorption of some amino acids onto paper to lower the measurement but this effect is considered minor.

3.2.2. Green tea

The mean content of amino acids in six green teabags was 2.28% and, in two green leaf teas it was 3.44% (Table 1), both much higher than the corresponding values found for black teas. Although the result of this study of teas from Australian supermarkets suggests that the content of amino acids in green teas is generally higher than that in black teas, green tea is usually manufactured using *C. sinensis* var. *sinensis*, whereas for black tea *C. sinensis* var. *assamica* is used (Hara et al., 1995). Amino acids and their infusion were found to vary from clone to clone of teas (Yao et al., 1992), so, meaningful comparison of the contents of amino acids between green and black teas becomes very complicated.

3.2.3. The solubility or permeability

The solubility (permeability) of amino acids from teabags ranged from 78.8% to 122%, with a mean of 94.2% (Table 2), which again shows great variability, reinforcing what was stated in 3.1.3.

3.3. Water extract

3.3.1. Black tea

According to the international standard, water extract of a tea is ''the soluble matter extracted from a test portion by boiling water, under the conditions specified in this international standard, expressed as a percentage by mass on a dry basis'' (ISO 9768, 1994). In this study, the water extract of eighteen black leaf teas ranged from 31.50% to 39.85%, with a mean of 36.20% (Table 1). Water extract in tea should be more than or equal to 32% of the dry mass (ISO 3720, 1986). The water extract of one of the 18 black leaf teas did not meet this standard.

Compared with the water extract of black leaf teas produced in other countries (Owuor et al., 1986), China 36.79%, India 36.89–41.95%, Sri Lank 36.72–46.90%, and Kenya 44.12%, it can be seen that the mean content of water extract in the black leaf teas from Australian markets is relatively low. In the three Australian-made black leaf teas, the water extract averaged 35.42%, which is lower than the overall mean of black leaf teas (36.20%) from Australian markets. In Australian-made black teabags, the average content was 36.97%, also lower than the overall mean of thirty black teabags from the market (37.39%). These results show that the water extract of Australian-made black leaf tea meets the international basic requirements, but, even so it was lower than the contents of the black teas produced in the countries which produce teas of traditional good quality.

3.3.2. Green tea

The mean content of water extract in six green teabags was 39.18% and, in two green leaf teas, it was 32.84% (Table 1), with the former being much higher than the content found in the black teabags and the latter being much lower than that of black leaf teas. The higher amount of water extract in green teas than in black teas may be due to differences in manufacture, leading to the decomposition of tea components to different degrees (Hara et al., 1995; Harbowy & Balentine, 1997). However, if marked variations occur in the same tea, it could be caused by inappropriate storage or too long a storage, which also causes the decomposition and/or deterioration.

It should be noted that one of the two green leaf teas showed a water extract even lower than 32%, implying that the quality of green leaf tea in Australian markets should be cause of deep concern.

3.3.3. The solubility or permeability

The permeability of water extract from teabags ranged from 83.43% to 99.27%, with a mean of 91.52% (Table 2), while green teabags averaged 93.95% and Australian-made black teabags averaged 89.06%. These results showed a lower variable profile than the caffeine or the amino acids in black teas. The very low level of the overall permeability of water extracts from the teabags, with the Australian-made teabags giving the lowest, again suggests that the quality of paper used for the manufacture of teabags for Australian markets is very variable and needs to be improved.

3.4. Moisture content and weight range of teabags

Moisture content is an important quality parameter of teas (Roberts & Smith, 1963) and is usually neglected by researchers, but not by the industries or tea traders. Tea researchers (Othieno & Owuor, 1984; Robinson & Owuor, 1993) suggested that the moisture content of the teas should be controlled to lie under 6.5% for marketing teas, whereas Millin (1987) noted that teas had a moisture content of 7–8% during retailing. In this study, 70% of the 56 tea samples from Australian supermarkets showed a moisture content above 6.5%, 30% of tea samples being above 7.0%. This result means that the moisture content of teas in Australian markets is high and produces negative effects on shelf life. In addition, it was noted, in this study, that four out of 34 teabags had a weight more than 10% different from the weight claimed. Such differences in weight in teabags could affect the storage and tea quality on the shelf, suggesting a need for improved quality control for the packaging of teabags in Australian markets.

4. Conclusion

The caffeine contents of black leaf tea (3.89%) and teabags (3.87%) from Australian supermarkets are close

to those found in the green leaf tea (3.71%) and teabags (3.83%). These contents are generally higher than the caffeine levels detected in the teas produced in the main tea producing countries. In contrast, total amino acids and water extract in the teas from the Australian markets are lower than the levels detected in the teas from markets of other countries. Furthermore, the moisture content of the teas in Australian markets is too high and so may affect tea quality during storage and retailing. The permeability of tea components from the teabags is an important parameter in assessing the quality of paper in relation to the quality of the teas. In this study, the low level of the permeability of caffeine, amino acids and water extractable components may affect the quality of the teas from Australian markets. Based on these analytical results and on comparing them with the findings of previous studies, the quality of the teas from Australian markets seems to be at an average world level.

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