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Phenolic compounds in tea from Australian supermarkets

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Abstract

Phenolic compounds constitute 50–70% of tea water extract and are the main quality parameters for teas. Theaflavins (TF), thearubigins (TR) and theabrownins (TB) are the major polyphenols that determine the quality of black tea. These compounds were measured in 56 leaf teas and teabags sampled from Australian supermarkets in Queensland. The various quantities of TF, ranging from 0.29% to 1.25%, indicate a quality difference that exists among the teas studied. Low TF content in black tea may be due to over-fermenting and/or long periods of storage. The solubility of TR and TB from teabags ranged from 82% to 92%, indicating that the permeability of teabags was variable. Variable quantities of TF in Australian teas show instability and a tendency of TF to oxidize during storage. Total polyphenols in green teas ranged from 14% to 34%, indicating a large variation, which was not reflected in price. The solubility of total polyphenols from teabags has been proposed as a useful quality index of the filtering paper used for the teabags. This chemical analysis of phenolic compounds in commercial teas may be a potential tool for the quality control of Australian manufactured and imported teas in Australian markets.

Keywords: Tea; Polyphenols; Theaflavins; Thearubigins; Theabrownins; Quality

1. Introduction

The major proportion (ca. 90%) of total phenolic compounds in tea is comprised of flavonoids, from green tea catechins (flavanols and flavanol gallates) to their complex oxidation products, theaflavins (TF) and thearubigins (TR, mistakenly presented as thearubigens in some literature) in black tea (Harbowy & Balentine, 1997; Lakenbrink, Lapczynski, Maiwald, & Engelhardt, 2000). TF and TR are soluble in hot water and represent 20–35% (w/w) of dry tea, from which about 45% of the tea constituents can be infused into hot water (Xiao, 1994). Tea phenolic compounds, known as tea polyphenols (Harbowy & Balentine, 1997), previously called tea tannins (Bokuchava & Skobeleva, 1969), have been regarded as the quality parameters or indicators of tea (Deb & Ullah, 1968; Ding, Kuhr, & Engelhardt, 1992; Obanda, Owuor, & Njuguna, 1992). In particular, theaflavins (TF) were used to assess the market value (Hilton & Ellis, 1972; Owuor, Reeves, & Wanyoko, 1986), clonal variations (Owuor & Obanda, 1995) and seasonal quality variations of black tea (Malec & Vigo, 1988). Thus, analysis of tea polyphenols is an effective method for the determination of tea quality.

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Traditional methods for the preparation and determination of polyphenols in fresh tea shoots or manufactured teas have been described by several researchers (Roberts, 1962; Roberts & Myers, 1958; Roberts & Smith, 1961). The most common methods are paper chromatography (Oshima & Nakabayashi, 1953a; Roberts, 1962; Roberts, Cartwright, & Oldschool, 1957; Roberts, Cartwright, & Wood, 1956), column chromatography (Oshima & Nakabayashi, 1953b; Whitehead & Temple, 1992) and colorimetric measurement (Muralidharan, 1997; Oshima & Nakabayashi, 1953c; Roberts & Smith, 1961). All those methods are based on either the oxidation or reduction properties of tea polyphenols. More recently developed analytical techniques are used to isolate, identify and determine individual polyphenolic compounds by HPLC (Harbowy & Balentine, 1997; Temple & Clifford, 1997; Yao & Nursten, 1997, 1998). However, colorimetric or spectrophotometric methods are still the most widely used, due to their simplicity for the determination of total phenolic compounds, TF and TR in tea (Harbowy & Balentine, 1997; Lakenbrink et al., 2000), particularly for tea industries.

Theaflavins, thearubigins and theabrownins are complex phenolic compounds deriving from the oxidation of catechins and their gallates during the fermentation stage of black tea processing (Roberts, 1962; Zhang, 1987). In the black tea trade, the contents of these compounds are measured as part of a regular quality control procedure. TF are the first stable oxidation products formed in tea fermentation, and refer to the intermediate compounds formed during the oxidation of catechins and catechin gallates. Theaflavins undergo further oxidation during fermentation, to form more polymerised thearubigins, and then condensed theabrownins, which are found to be polymerised thearubigins linked with proteins (Yuan, 1983; Zhang, 1987). TR are a group of compounds formerly recognised as insoluble fractions, SI and SII, of ethyl acetate extraction (Roberts et al., 1956). TR was further separated by butanol into soluble TR and insoluble TR (Yuan, 1983). Of those compounds, TF contributes the brisk and astringent taste and bright golden colour to black tea quality, while TR contribute the reddish colour and richness in taste, totally termed 'body' to black tea (Roberts, 1962). Therefore, both classes of compounds are associated with quality and other desirable liquor characters (Bhatia, 1960; Roberts, 1962). In contrast, TB endows tea liquor and leaf with a dark brown colour, which has a negative effect on tea quality (Yuan, 1983). Therefore, analyses of these three groups of phenolic compounds can be used as a objective methods in the determination of black tea quality.

Takeo and Oosawa (1976) studied the whole profile of phenolic compounds by comparing the price and sensory characteristics of black tea. However, no previous studies have been reported on the phenolic compounds in teas from Australian supermarkets. This is the first systematic survey of teas from Australian markets for the quantification of tea polyphenols, and could have potential for the quality assessment of teas in this market.

2. Materials and methods

2.1. Samples

Leaf tea and teabags, commercially available from three major supermarkets (Woolworths, Coles and Franklins) in Brisbane, Gatton and Toowoomba of Queensland, Australia, were randomly sampled and used for this study, except for one leaf tea that was manufactured in the laboratory from the fresh tea leaves obtained from commercial tea estates at Malanda in north Queensland. This leaf was used as a representative of fresh Australian manufactured teas for the supermarkets for a comparison in this study. One crude black tea sample provided by the same tea estate was used for the comparison.

Teabags were either heat-sealed, as in the UK-type, or double chamber US-type. The sampling method used in this study was based on the international standard method [ISO 1839 (BS 5987), 1980].

2.2. Moisture

Tea moisture was measured using a vacuum oven based on an international standard method [ISO 1573 (BS 6049-2), 1980].

2.3. Total phenolic compounds

2.3.1. General

The method was based on the description of tea-researchers (C. M. C., 1991; Roberts, 1962) with modification of the recent work (Muralidharan, 1997; Yao, Chen, & Cheng, 1993; Yao, Cheng, Chen, & Liu, 1992). Details of the method are as follows.

2.3.2. Preparation of the tea solution

Boiling water (200 ml) was added to 2 g leaf tea or 1 teabag (ca. 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 solution was filtered through cotton wool and the residue was washed with distilled water (3×10 ml). The tea solution was combined, then cooled to room temperature and finally diluted to 250 ml with distilled water. The tea solution was prepared in duplicate.

2.3.3. Tartrate solution

$FeSO_4$ (1 g) and $KNaC_4H_4O_6$ (5 g) were dissolved in distilled water and made up to 1000 ml.

2.3.4. Buffer solution

Na_2HPO_4 (23.377 g) was dissolved in distilled water (1000 ml) and KH_2PO_4 (9.078 g) was dissolved in distilled water (1000 ml). A buffer solution consisting of 85% (v/v) of Na_2HPO_4 solution and 15% (v/v) of KH_2PO_4 solution was prepared.

2.3.5. Measurement of total polyphenols

Tea solution (1 ml), distilled water (4 ml) and tartrate solution (5 ml) were placed in a volumetric flask and then diluted to 25 ml with the buffer solution. The mixture absorbance was measured using a Pharmacia Ultrospec III UV/Vis spectrophotometer at 540 nm.

2.3.6. Calculation

Total polyphenols (%) = $3.914E \times V_0 \times 100/1000/V_1/W = 0.3914EV_0/V_1/W$, where E is 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 ml) and W is the dry weight of the tea sample.

2.4. TF, TR and TB

2.4.1. General

The method was based on the description of various researchers (C. M. C., 1989; Roberts, 1962; Roberts & Smith, 1961; Zhang, 1987) with modifications of recent work (Harbowy & Balentine, 1997; Muralidharan, 1997; Yao et al., 1993). Details of the method used are as follows.

2.4.2. Extraction

Leaf tea (3 g) or 2 tea bags (ca. 4 g) were stirred with hot water (125 ml) at 90 °C for 10 min. After filtration, the tea solution was allowed to cool to room temperature. The extraction method was performed in duplicate.

2.4.3. Ea

A portion of the tea solution (30 ml) was mixed with ethyl acetate (30 ml) in a separating funnel and shaken for 5 min. Part of the ethyl acetate layer (2 ml) was diluted to 25 ml with 95% (v/v) ethanol. The absorption of this ethanol solution was recorded as Ea.

2.4.4. Eb

A portion of the original tea solution (15 ml) was mixed with *n*-butanol (15 ml) and shaken for 3 min. Part of the aqueous layer (2 ml) was mixed with saturated oxalic acid (2 ml) and distilled water (6 ml) and then diluted to 25 ml with 95% (v/v) ethanol. The absorption of this ethanol solution was recorded as Eb.

2.4.5. Ec

Another portion (15 ml) of the ethyl acetate layer was mixed with 2.5% (w/v) $NaHCO_3$ (15 ml) and shaken for 30 s. The aqueous layer was discarded and part of the ethyl acetate layer (4 ml) was diluted to 25 ml with 95% (v/v) ethanol. The absorption of this ethanol solution was recorded as Ec.

2.4.6. Ed

A portion of the aqueous layer of Ea (2 ml) was mixed with saturated oxalic acid (2 ml) and distilled water (6 ml), and then diluted to 25 ml with 95% (v/v) ethanol. The absorption of this ethanol solution was recorded as Ed.

2.4.7. Measurements of TF, TR and TB

The absorbance of the above solutions were measured with a Pharmacia Ultrospec III spectrophotometer at 380 nm, with 95% (v/v) of ethanol as a blank.

2.4.8. Calculation

$$\begin{aligned} \text{TF}\% &= 2.25 \times \text{Ec}/(1 - M), \\ \text{TR}\% &= 7.06 \times (2\text{Ea} + 2\text{Ed} - 2\text{Eb} - \text{Ec})/(1 - M), \\ \text{TB}\% &= 7.06 \times 2\text{Eb}/(1 - M), \end{aligned}$$

where Ea, Eb, Ec and Ed are the corresponding (absorbance) readings from the spectrophotometer of the above solutions. M is the moisture content of the tea sample. If the sample was not 3 g, the calculations were multiplied by 3 in the formula and then divided by the actual weight of the sample analysed, because this empirical formula is based on a 3 g basis. The percentage of each compound was calculated on a dry weight basis (w/w).

3. Results and discussion

3.1. Total polyphenols

Total polyphenols of black tea include some residues of green tea polyphenols that were not oxidised during the processing, and the oxidation products of polyphenols such as TF. The content of total polyphenols in black tea leaf was between 14% (w/w) and 20% (w/w), with an average of 17% (w/w). This was similar to the total polyphenols detected in black teabags, which ranged from 13% to 27% (w/w), with an average of 18% (w/w). The three Australian-grown and manufactured black leaf teas analysed in this study had the lowest content of total polyphenols [14% (w/w)], while the total polyphenols detected in Australian black teabags averaged 16%, which is less than 18% of the other black teabags sourced from the supermarkets. The results may suggest that Australian tea providers prefer teas with a slight over-fermentation that allows more polyphenols to be oxidised. However, Australian grown and manufactured tea represents only 10% (w/w) of the teas consumed in Australia each year (Chudleigh, 1999). Thus, the total polyphenols in the imported black teas, including black leaf tea and tea bag, represent the major coverage of these phenolic compounds in the teas consumed in Australia.

Sanderson (1972) suggested that polyphenols in black tea were comprised of simple (unoxidsed) polyphenols (5%, w/w) and oxidised polyphenols (25%, w/w). Most of those polyphenols can be infused into tea brew, with simple polyphenols being 4.5% (w/w) and the oxidised ones being 15% (w/w), of total water soluble dry mass in the black tea. Thus, the content of total polyphenols in black tea is 19.5% on a dry weight basis. In Kenya, Obanda, Owuor, and Taylor (1997) reported that the concentration of total polyphenols in black tea is about 20%, including 5% simple polyphenols, 2% TF and 13% TR while, in India, Dev Choudhury and Goswami (1983) measured a large number of black teas using the same method and found a mean value of 20% total polyphenols (dry weight basis) in the teas. Therefore, the content of total phenolic compounds in the imported black teas from Australian markets is close to that of black teas produced in the above countries, but the phenolic content in Australian-grown and manufactured black teas is less than that of the imported teas. Such a result may indicate that Australian black tea is over-fermented during the manufacturing process.

Total polyphenols of green teabags ranged from 21% to 33%, with an average of 25%. This was much higher than the polyphenolic content of black tea bags, with an average of 18%. This is because some of the green tea polyphenols are oxidised during the fermentation stage of tea processing. It is possible that some of the oxidation products, although still phenolic compounds, could not be detected by the method used, e.g. the more polymerised compounds, such as part of the polymerised TR.

The differences in polyphenol content among the black teas and among the green teas were mostly due to differences in their botanical origins. Usually, teas originating from Indian or Sri Lankan varieties (*Camellia sinensis* var. Assamica) have higher polyphenol contents (ca. 30%) than those from China variety (*Camellia sinensis* var. Sinensis, ca. 20%) (Hara, Luo, Wickremasinghe, & Yamanishi, 1995; Harbowy & Balentine, 1997). In this study, the total polyphenols of two green teabag samples originating from Sri Lanka were 26% and 34%, respectively, whereas the polyphenols of three green teabag samples originating from China were 21%, 21% and 23%, respectively. Thus, under such circumstances, the polyphenol content can be used as an index of the botanical origin of a tea, and/or a quality parameter for tea, together with the other quality parameters, such as tea flavour index (Owuor, Horita, Tsushida, & Murai, 1987), amino acid (Hara et al., 1995) and caffeine contents (Owuor & Chavanji, 1986).

According to the above findings, it is seen that the low contents of phenolic compounds among teas of the same botanical origin may indicate a long period of storage for both black and green teas, or over-fermentation for black teas. In this situation, polyphenols could be used as one of the major quality indicators. In this study, one imported green leaf tea sample was found to contain only 15% of total polyphenols. Further examination of the contents of TF, TR and TB in this particular green tea showed that these oxidation compounds were present in green tea in equal amounts to those in normal black teas. Since TF, TR and TB are oxidation products and generated during fermentation and storage, they should not be present in high quality green tea. Their presence in higher amount in a particular green tea suggests that deterioration occurred to this green tea during either processing or storage. The sensory evaluation confirmed that this tea might have been stored too long to be drinkable. These results suggest that analysis of phenolic compounds of teas under specific conditions (i.e., varieties and types) could indicate the processing and storage defects, with resultant assessment of the quality of a tea.

A comparison of the polyphenols released into hot water from tea, with or without a bag, showed that more polyphenols were extracted from green tea bags (ca. 95%) in comparison to green leaf tea than from black tea bags (ca. 91%) in comparison to black leaf tea, whereas Australian black teabags showed the lowest solubility with an average of 78.48% (Table 1). Again, this may be due to the fact that part of the more polymerised TR of black teas could not be measured and/or due to the variations of tea clones used for blending the commercial teas. In general, green teas contain relatively simple polyphenols (mainly catechins and their gallates), so it is relatively simple to measure the total polyphenol content. In addition, the types of additives used in the processing of teabags might affect the permeability of the filter papers. Thus, the percentage of the polyphenols infused in a brew from the teabags could be a useful index of the quality of the teabags. However, no total polyphenol content in teabags is available for a comparison.

3.2. Theaflavins, thearubigins and theabrownins

The mean contents of theaflavins of 30 kinds of teabags and 15 black leaf tea samples were very similar, with 0.77% in teabags and 0.75% in leaf teas. The

Table 1
Solubility of total polyphenols in green and black teabags

Type	Without bag (%, A)	With bag (%, B)	Ratio (%, B/A)
Green tea ($n = 6$)	25.25	24.13	95.37
Black tea ($n = 29$)	17.93	16.34	90.91
Aus black tea ($n = 5$)	17.17	14.26	78.48

Table 2
Mean TF, TR and TB contents (%) in black teas

Tea	Level	TF	TR	TB
Teabag (*n* = 38)	Highest	1.25	12.1	11.4
	Lowest	0.29	5.33	7.61
	Average	0.77	8.90	9.77
Leaf (*n* = 16)	Highest	1.10	10.7	10.5
	Lowest	0.32	3.91	7.32
	Average	0.75	7.61	8.66

contents of TR and TB in teabags were higher than their contents in leaf teas (Table 2). Further analysis found that content of TF in the teabags ranged from 0.29% to 1.25%, which appeared to be more variable than leaf teas which had a range of 0.32–1.10%. This study found that three of the commercial teas, available in Australian supermarkets were processed for special market needs, and showed low levels of TF. During the processing of these teas, the producer might add some flavourings and additives (which are claimed on the label and can be identified by sensory evaluation) that resulted in low theaflavin contents due to oxidation.

Using the same spectrophotometric method, Roberts and Smith (1963) found that when the content of TF in a commercial tea reached 1.63%, tea tasters' remarks were "very bright liquor and rich colour," which indicated a very good quality black tea. Another study by Owuor and Obanda (1995), in Kenyan commercial black teas, reported that the total TF ranged from 1.80% to 2.59%, with an average of 2.14%. This level of TF is much higher than that of the teas available in Australia, indicating that most of the imported teas were either over-fermented black tea or underwent a relatively long period of storage that resulted in further oxidation or decrease of TF.

During the phenolic analysis of green tea samples, it was found that the aroma of all the samples had "gone off", with an unpleasant aroma characteristic of long storage. To confirm this finding, TF, TR and TB were measured as for black teas, though those compounds are supposed to be absent from green teas. All the analysed teas were teabags, except for one leaf tea sample. Their mean contents of these compounds were 0.24% TF, 6.96% TR and 5.28% TB, respectively (Table 3). These results showed that all the green teas analysed had undergone serious oxidation, indicating that the teas were stored too long to be fresh. Even though they were still drinkable under these conditions, they had no green tea taste at all. However, the green leaf tea sample smelled stale during analysis, based on the content of these three black tea components.

Analysis of the fresh black tea, collected from the Australian tea factory, showed that this fresh tea contained 0.76% TF, 9.24% TR and 9.83% TB. Since this sample was collected just after processing, and was going to be sorted as leaf teas and/or teabags, it was considered as a representative of Australian manufactured black teas. On comparing its contents of TF, TR and TB with the other samples from Australian supermarkets (Table 2), it was found that this Australian black tea had similar contents of these compounds to those in the black teas from the supermarkets. In early studies, it has been found that good quality black teas with appropriate fermentation should have a theaflavin content of more than 1%, with corresponding thearubigin content more than 10% (Bhatia, 1960; Roberts, 1962). Thus, the results of this study revealed that this Australian black tea was over-fermented as a crude black tea because of its low theaflavin content (0.76%). Cloughley (1981a, 1981b) has shown that black tea continues to ferment (involving further oxidation of TF) just following the completion of whole tea manufacturing, which is the so-called post-fermentation or post-mature process. In fact, oxidation of phenolic compounds occurs in all types of teas during the storage period (Hara et al., 1995). Therefore, it is always recommended that fresh black teas should normally have a higher content of TF (TF > 1%), a higher content of TR (TR > 10%), and an adequate TF/TR ratio (>0.1).

Roberts (1962) compared the TF and TR contents in teas sampled from India with sensory evaluation by well-trained tea tasters. It was found that a high TF content, in conjunction with high TR content, indicated high quality black tea, such as that of the TF/TR ratio of 3 tea samples analysed by Roberts in Table 4. In this current study, eight imported black teas were compared with the black tea from Australian markets (Table 4). Results show that the tea from Australian supermarkets has the lowest TF content and the lowest TF/TR ratios. Accordingly, the black tea from Australian markets

Table 3
TF, TR and TB contents (%) in green tea

Sample code	103	104	111[a]	115[b]	134	140	Mean	Standard deviation
Origin	Ceylon	Ceylon	Mixed	Blended	China	China		
TF	0.18	0.18	0.15	0.15	0.18	0.62	0.24	0.19
TR	7.44	8.62	6.60	6.19	7.42	5.48	6.96	1.11
TB	5.22	3.99	5.04	5.95	3.53	7.94	5.28	1.57
Total polyphenols	26.2	33.9	21.4	21.7	23.2	14.8	23.5	6.30

[a] *Mixed*: Australian manufactured teas mixed with imported teas.
[b] *Blended*: Blended imported teas.

Table 4
Comparison of TF, TR and TF/TR in black teas from Australian markets with overseas studies (based on a similar analytical method)

Market	TF (%)	TR (%)	TF/TR
Australia[a]	0.77	8.89	0.09
UK[b]	1.54	11.09	0.14
Continental Europe[b]	1.15	11.56	0.10
Middle East[b]	1.47	12.18	0.12
USA[b]	1.17	9.45	0.12
India[c]	2.21	16.0	0.14
Roberts[d] (Indian tea)	1.10	10.3	0.11
	1.55	15.9	0.10
	1.75	15.4	0.11

[a] The values entered here are averages from the imported and Australian manufactured black tea.
[b] Adapted from Lakenbrink et al. (2000).
[c] Adapted from Bhatia (1960).
[d] Adapted from Roberts (1962).

should have a relatively softer taste than those consumed overseas because of the lower TF contents and lower TF/TR ratios. Some of the imported tea samples analysed in this experiment showed higher TF and TR contents and also similar TF/TR ratios to the overseas counterparts. This implies that teas with high TF content have a market in Australia. In other words, some Australians select strong-tasting or high TF-content teas, as do overseas consumers. Thus, this result could be very important to the Australian tea industry and international tea traders in profiling the Australian tea market and the diverse demands of its consumers.

The price of the tea samples was also compared with their TF and TR contents, as well as the TF/TR ratio. However, there were no direct relationships. This may be because the price of the teas on markets was, not only based on tea quality, but also on packaging, re-processing, and blending of the teas. However, the contents of TF, TR and ratio of TF/TR could constitute a useful index of quality for tea exporters and importers (Takeo & Oosawa, 1976). As they are directly associated with the black tea quality, the contents of TF, TR and ratio of TF/TR could also be used by the tea producers to monitor the processing of black tea.

The solubilities of TR and TB from teabags were from 82% to 86%, and from 83% to 92%, respectively. These results indicate that the permeability of teabags was variable. The variation of TF solubility from teabags was even larger, ranging from 75% to 103%, suggesting that TF is unstable due to its tendency to be oxidized, as discussed earlier. Teabags are mainly prepared with a view to quick solubility and providing convenience for consumers. Thus, the tea components of teabags should be soluble in hot water in a very short time. The results of the permeability of these phenolic compounds from black teabags suggest that the quality of the filter used for the teabags needs to be reviewed.

4. Conclusion

Total polyphenols of black leaf tea (17%) and teabags (18%) from Australian markets are close to those in the black tea produced in other countries. In green teas from the Australian markets, total polyphenols are variety-dependent, with var. Assamica ranging from 26% to 34% and var. Sinensis from 21% to 23%. The solubility of total polyphenols from green teabags (95%) is higher than that from black teabags (91%). Thus, the percentage of the polyphenols dissolved from teabags suggests that the quality of the filter papers used for teabags is variable. The results of this study may indicate that total polyphenols could be used as one of the quality indicators for tea processing and marketing.

TF, TR and TB in the black teabags from Australian markets averaged 0.77%, 8.90% and 9.77%, respectively while, in the black leaf tea, they were 0.75%, 7.61% and 8.66%, respectively. TF, TR and TF/TR are very important quality indicators of black teas. Black teas from Australian markets had lower contents of TF and TR, as well as TF/TR ratios, than those from overseas markets. The price of black teas from markets was not related to the contents of TF, TR or TF/TR, although it was assumed to be by the tea producers. Similarly, the price of green teas from the markets showed the same trend. In addition, different solubilities of TF, TR and TB from black teabags suggested that the quality of the filter paper for the black teabags is variable. Furthermore, large amounts of TF, TR and TB found in the green teas from Australian supermarkets strongly indicated a long and/or unsuitable storage of the teas, resulting in no green tea properties. Thus, it can be concluded that the quality of teas in the Australian markets is very variable, dependent on the source of import areas, types and places of packaging, and variety and types of teas.

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