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# Influence of coffee blends and roasting process on the antioxidant activity of coffee

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## Abstract

The aim of this paper is to develop specification for the production of roasted coffee with improved nutritional quality. In this respect, the influence of different coffee blends and temperatures of roasting on the antioxidant activity of coffee was examined. Also, the composition of some bioactive components in the analyzed samples was determined. Two Arabica coffees of different quality and Robusta coffee were used for the preparation of coffee blends. Experimentally determined green coffee blends B1, B2 and B3 were roasted at 167°C, 171°C and 175°C for 25 minutes in order to establish the effect of roasting temperature on the antioxidant activity and the content of bioactive components in blends. Based on the results of DPPH and ABTS test, coffee blends B3 and B2, roasted at 171°C, had the highest ( $P < 0.05$ ) antioxidant activity. Those coffee blends had higher content of Robusta coffee than coffee blend B1. Results of this research can provide opportunities for the development of roasted ground coffee with improved nutritive quality, which could ensure competitiveness of producers in the target market.

**Keywords:** coffee blends, antioxidant activity, nutritive quality.

## 1. INTRODUCTION

In recent years, consumers are paying more attention not only to the sensory, but also to the nutritional quality of food products, as well as to the impact of food on their health. Health-promoting aspects of foods are recognised as a trend for the food industry to work on and it is related to food processing and components (Sijtsema, Backus, Linnemann, & Jongen 2009). The quest of knowledge regarding coffee quality is spreading due to the growing appreciation of this worldwide popular beverage (Noor Aliah, Fareez Edzuan, & Noor Diana 2015). Arabica and Robusta coffee species have the highest commercial importance. Arabica has a much more sophisticated sensory quality and it is more expensive than Robusta. On the other hand, Robusta has the advantage of allowing extraction of large amounts of soluble solids, which enables its use in blends (Mussatto, Machado, Martins, & Teixeira 2011; Sacchetti, Di Mattia, Pittia, & Masrocchio 2009). Consumption of coffee beverage, which is prepared by adding roasted ground coffee to the tap water

at the moment of boiling and heating it until the moment rich foam starts rising, is characteristic for Southeastern Europe. For the preparation of this type of coffee beverage, blends of Arabica and Robusta mixed in different proportions and roasted at different temperatures depending on the raw materials and production conditions, are usually offered on the market (Odžaković, Džinić, Mirković-Grujić, Kravić, & Jokanović 2015).

Recently, scientific studies have pointed out the positive effect of coffee on human health because coffee is one of the products rich in biologically active compounds. Antioxidant activity of coffee is affected by the green bean composition and processing methods (Farah & Donangelo 2006; Marcucci, Dias, Almeida, & Benassi 2017). Recent interest in food phenolics has increased greatly because of their antioxidant, free radical-scavenging abilities and potential effects on human health (Pandey & Rizvi 2009). Flavonoids represent the most common and widely distributed group of plant phenolics. Among the flavonoids, flavones, flavonols, and their glycosides are the most com-

**Table 1.** Blends of different coffee species and time-temperature regime of roasting process

Blends	Arabica 1 <sup>st</sup> class: Arabica 2 <sup>nd</sup> class: Robusta	Parameters of roasting	
		T (°C)	t (min)
B1	52.00%:34.00%:14.00%	167	25
		171	
		175	
B2	38.00%:38.00%:24.00%	167	25
		171	
		175	
B3	46.00%:30.00%:24.00%	167	25
		171	
		175	

mon. Phenolic compounds are highly unstable during food processing (Farah & Donangelo 2006; Ifie & Marshall 2018). Roasting of coffee represents a temperature-time dependent process that affects the chemical and physical transformations of coffee beans. Several studies indicated that a high content of polyphenols in coffee plays an important role in its strong antioxidant action (Cämmerer & Kroh 2006).

The aim of this paper is to develop specification for the production of roasted coffee with improved nutritional quality. In this respect, the influence of different coffee blends and temperatures of roasting on the antioxidant activity of coffee was examined. Also, the composition of some bioactive components in the analyzed samples was determined.

## 2. MATERIAL AND METHODS

Blends of coffee were prepared from Arabica 1<sup>st</sup> class (maximum 8 black beans/300g of sample and large size of beans) Rio Minas from Brazil, Arabica 2<sup>nd</sup> class (maximum 19 black beans/300 g of sample and medium size of beans) Rio Minas from Brazil and Robusta Cherry from India. All samples were obtained from a local manufacturer. The roasting process was performed in industrial conditions, in a roaster which has a capacity of 150 kg/batch, at three different temperatures. The plan of experiment is presented in Table 1.

### 2.1. Coffee extracts preparation

The coffee extracts were prepared according to a procedure described by Pérez-Hernández, Chávez-Quiroz, Medina-Juárez, and Gámez Meza (2012) with some modifications. Samples of green and roasted ground coffee were extracted with hot deionised water (90°C) at a coffee/solvent ratio 0.5/100 for 5 min with constant stirring. After extraction, the extracts were centrifuged for 5 min

at 2000 rpm. The extraction was repeated three times for each sample, using the previously described method. The extracts were stored at a temperature of -20°C until analysis.

### 2.2. Determination of total phenol content

The total phenol content (TPC) of each coffee extract was determined spectrophotometrically (UV/Vis spectrophotometer PerkinElmer Lambda 25), following Folin-Ciocalteu procedure, according to the Wolfe, Wu, and Liu (2003) modified method. The reaction mixture consisted of 1.5 mL of Folin-Ciocalteu solution (stock Folin-Ciocalteu solution dissolved with water in 1/10 ratio) and 1.5 mL of 7.5% NaHCO<sub>3</sub> added to 0.2 ml of diluted extract. The absorbance was measured after 30 min storing (in a dark place at room temperature) at 765 nm, along with the blank. The content of the total phenols in the samples was determined based on the calibration curve ( $A = 2.543C + 0.022$ ;  $R^2 = 1.0000$ ), defined on the basis of the measured values of the absorbances and the known values of the chlorogenic acid concentration (50, 100, 200, 300, 400 µg/ml). The calibration curve ( $A = 0.0043C - 0.0247$ ;  $R^2 = 0.9954$ ) was defined with the use of gallic acid as a standard (50, 100, 150, 200, 250 µg/ml). The results were expressed as mg chlorogenic acid equivalents (CGA)/g of coffee sample and as mg gallic acid equivalents (GAE)/g of coffee sample.

### 2.3. Determination of total flavonoid content

The total flavonoid content (TFC) was determined according to method of Ordonez, Gomez, Vattuone, et al. (2006). The reaction mixture of 1 ml of 2% AlCl<sub>3</sub> ethanolic solution and 1 ml of diluted extract was prepared. The absorbance was measured after 30 min storing (at dark place) at 420 nm, along with the blank. Based on the calibration curve ( $A = 0.032C + 0.0445$ ;  $R^2 = 0.9980$ )

defined on the basis of measured values of absorbances and known values of concentration of quercetin solution (10-80  $\mu\text{g}/\text{mL}$ ), the content of total flavonoids in the samples was determined and the results were expressed as mg quercetin equivalents (QE)/g of coffee sample.

#### 2.4. Determination of flavonol content

The flavonol content (FC) was determined according to method of Kumaran and Karunakaran (2007). The reaction mixture consisted of 1 mL of 2%  $\text{AlCl}_3$  ethanolic solution and 1.5 mL (50 g/L) sodium acetate solutions added to 1 mL of diluted extract. The absorption at 440 nm was read after 2.5 h storing in a dark place. Based on the defined calibration curve ( $A = 0.0228C + 0.0345$ ;  $R^2 = 0.9996$ ), the flavonol concentration in the samples was determined. The concentration of quercetin for the determination of the calibration curve ranged from 10-80  $\mu\text{g}/\text{mL}$ . FC was calculated and expressed as mg quercetin equivalents (QE)/g of coffee sample.

#### 2.5. Determination of antioxidant activity by DPPH method

The effect of coffee extract on DPPH radical (2,2-diphenyl-1-picrylhydrazyl) was determined by the documented method (Liyana-Pathirana & Shahidi 2005). The reaction mixture of 1 ml of 0.135 mM DPPH methanolic solution and 1 ml of diluted extract was prepared. The mixture was left in a dark for 30 min and absorbance was measured at 515 nm, along with the blank. The calibration curve ( $A = 2019.5C + 1.3948$ ;  $R^2 = 0.9994$ ) was constructed at a concentration of Trolox 0.005; 0.01; 0.02; 0.04  $\mu\text{mol}/\text{mL}$ . The results were expressed as Trolox equivalents  $\mu\text{mol}$  (TE)/g coffee sample.

#### 2.6. Determination of antioxidant activity by ABTS method

The Trolox equivalent antioxidant capacity (TEAC) of coffee extracts was estimated by the ABTS radical cation (2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)) decolourisation assay (Re et al. 1999). The ABTS radicals were generated by reacting ABTS aqueous solution (7 mM) with  $\text{K}_2\text{S}_2\text{O}_8$  (2.4 mM) stored in a dark place for 16 h. The working solution was obtained by diluting the stock solution of the ABTS radical cation with methanol to obtain an absorbance of  $0.7 \pm 0.005$  at 734 nm. The reaction mixture of 1 ml of ABTS solution and 1 ml of diluted extract was prepared. The absorbance was measured after 7 min storing (at dark place) at 734 nm, along with the blank. The calibration curve ( $A = 4515C + 0.1882$ ;  $R^2 = 0.9934$ ) was constructed at a concentration of Trolox

0.003; 0.005; 0.010; 0.012; 0.016  $\mu\text{mol}/\text{mL}$ . The results were expressed as Trolox equivalents in  $\mu\text{mol}$  (TE)/g coffee sample.

#### 2.7. Statistical analysis

All measurements were carried out in triplicate, and presented as mean  $\pm$  standard deviation (SD). All results were subjected to the one-factor analysis of variance (ANOVA) and when applicable Duncan's test was performed to estimate the significance of differences between mean values at  $P < 0.05$ , using Statistica 12.0 software (StatSoft, Inc., Tulsa, OK, USA). Correlations between TPC, TFC, FC and antioxidant activity (DPPH, ABTS) were assessed by Pearson's correlation coefficient, and its significance by  $t$ -test for  $P < 0.05$ .

### 3. RESULTS AND DISCUSSION

Table 2 presents the results of total phenol content presented as chlorogenic acid equivalent (mg CGA/g) and gallic acid equivalent (mg GAE/g), total flavonoid content, flavonol content and antioxidant activity (DPPH, ABTS) of green and differently roasted coffee blends experimentally determined (Table 1). Chlorogenic acid was used as a standard for determination of the total phenolic content, as well as commonly used gallic acid, since the coffee has a high content of this phenolic component. Results showed that total phenol content (mg CGA/g and mg GAE/g) was the highest in green coffee blends, and the lowest in coffee blends roasted at 175°C, which means that TPC decreases during the roasting process. TPC in green coffee blends and coffee blends roasted at 167°C, 171°C and 175°C were statistically different ( $P < 0.05$ ). Total flavonoid content and flavonol content were significantly higher ( $P < 0.05$ ) in coffee blends roasted at 175°C compared to TFC and FC in coffee blends roasted at 167 and 171°C and in green coffee blends. Results showed that TFC and FC increased during the roasting process. The results are in accordance with literature data (Odžaković, Džinić, Kukrić, & Grujić 2016; Pérez-Hernández et al. 2012; Trandafir, Nour, & Ionica 2013). Results showed that the highest TPC was in blend B3 (Arabica 1<sup>st</sup> class 46.00%: Arabica 2<sup>nd</sup> class 30.00%: Robusta 24.00%) for green and coffee blends roasted at 171 and 175°C. Coffee blend B2 (Arabica 1<sup>st</sup> class 38.00%: Arabica 2<sup>nd</sup> class 38.00%: Robusta 24.00%) had the highest TPC for coffee blends roasted at 171°C. Blend B1 (Arabica 1<sup>st</sup> class 52.00%: Arabica 2<sup>nd</sup> class 34.00%: Robusta 14.00%) had the lowest TPC, TFC and FC. Robusta coffee contains a higher concentration of phenolic compounds, compared to Arabica coffee (Fărcaș et al. 2014; Gichimu, Gichuru, Mamati, & Nyende 2014; Odžaković et

**Table 2.** Content of total phenol, total flavonoid and flavonol and antioxidant activity of green coffee blends and coffee blends roasted at 167, 171 and 175°C

Blends	<i>T</i> (°C)	TPC (mg CGA/g)	TPC (mg GAE/g)	TFC (mg Q/g)	FC (mg Q/g)	DPPH (μmol TE/g)	ABTS (μmol TE/g)
B1	0	53.85 <sup>a</sup> ±0.44	33.46 <sup>a</sup> ±0.42	3.32 <sup>c</sup> ±0.16	1.32 <sup>c</sup> ±0.04	191.06 <sup>d</sup> ±0.70	195.92 <sup>c</sup> ±0.03
	167	47.58 <sup>b</sup> ±1.44	30.31 <sup>b</sup> ±1.29	4.28 <sup>b</sup> ±0.12	1.94 <sup>b</sup> ±0.01	211.03 <sup>b</sup> ±2.29	226.61 <sup>a</sup> ±3.21
	171	43.79 <sup>c</sup> ±1.01	28.03 <sup>c</sup> ±0.71	4.47 <sup>b</sup> ±0.07	2.06 <sup>b</sup> ±0.16	235.57 <sup>a</sup> ±2.90	217.62 <sup>b</sup> ±2.86
	175	38.44 <sup>d</sup> ±1.36	25.16 <sup>d</sup> ±0.67	4.72 <sup>a</sup> ±0.06	2.35 <sup>a</sup> ±0.06	197.82 <sup>c</sup> ±1.03	198.75 <sup>c</sup> ±0.82
B2	0	55.36 <sup>a</sup> ±1.27	34.46 <sup>a</sup> ±1.04	3.47 <sup>c</sup> ±0.06	1.36 <sup>c</sup> ±0.06	197.28 <sup>c</sup> ±1.29	193.50 <sup>d</sup> ±1.79
	167	49.41 <sup>b</sup> ±0.95	31.39 <sup>b</sup> ±1.64	4.43 <sup>b</sup> ±0.07	1.99 <sup>b</sup> ±0.06	220.41 <sup>b</sup> ±1.03	233.55 <sup>b</sup> ±2.79
	171	44.88 <sup>c</sup> ±0.83	29.34 <sup>c</sup> ±0.41	4.57 <sup>b</sup> ±0.11	2.10 <sup>b</sup> ±0.07	240.38 <sup>a</sup> ±2.44	240.81 <sup>a</sup> ±0.54
	175	39.39 <sup>d</sup> ±1.49	25.74 <sup>d</sup> ±0.51	4.87 <sup>a</sup> ±0.26	2.45 <sup>a</sup> ±0.10	200.28 <sup>c</sup> ±1.57	199.73 <sup>c</sup> ±1.94
B3	0	55.49 <sup>a</sup> ±1.18	34.47 <sup>a</sup> ±1.22	3.48 <sup>d</sup> ±0.08	1.37 <sup>d</sup> ±0.05	196.06 <sup>d</sup> ±1.34	194.83 <sup>d</sup> ±1.10
	167	49.21 <sup>b</sup> ±0.93	31.27 <sup>b</sup> ±1.19	4.42 <sup>c</sup> ±0.09	1.98 <sup>c</sup> ±0.05	221.08 <sup>b</sup> ±1.06	233.86 <sup>a</sup> ±2.80
	171	45.00 <sup>c</sup> ±0.95	29.04 <sup>c</sup> ±0.76	4.58 <sup>b</sup> ±0.05	2.10 <sup>b</sup> ±0.05	240.63 <sup>a</sup> ±2.87	225.47 <sup>b</sup> ±2.25
	175	39.59 <sup>d</sup> ±1.62	25.81 <sup>d</sup> ±0.27	4.86 <sup>a</sup> ±0.06	2.44 <sup>a</sup> ±0.05	202.17 <sup>c</sup> ±0.55	201.65 <sup>c</sup> ±3.28

TPC – Total phenol content. TFC – Total flavonoid content. FC – Flavonol content. DPPH – 2,2-diphenyl-1-picrylhydrazyl. ABTS – 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid).

<sup>a-d</sup>Mean that values with different letters in the same colon are significantly different at  $P < 0.05$

al. 2016). The highest TFC was in coffee blend B3 (0°C and 175°C) and in coffee blend B2 (167°C and 171°C). The highest FC was in coffee blend B3 (0°C, 171°C and 175°C) and in coffee blend B2 (167°C). These results might be associated with literature data (Fărcaș et al. 2014; Hečimović, Belščak-Cvitanović, Horžić, & Komes 2011; Odžaković et al. 2016) according to which Robusta coffee contains a higher concentration of total flavonoids compared to Arabica coffee. Therefore, coffee blends B2 and B3, which contain a higher percentage of Robusta coffee (24.00%), had a higher content of total phenols, compared to the coffee blend B1, which contains a lower percentage of Robusta coffee (14.30%).

Based on the results of DPPH test, coffee blends B1, B2 and B3 had the highest antioxidant activity at 171°C, and the lowest at 0°C. Blends roasted at 171°C had significantly higher ( $P < 0.05$ ) antioxidant activity than green blends and blends roasted at 167 and 175°C. Green blends B1 and B3 had significantly lower ( $P < 0.05$ ) antioxidant activity than roasted coffee blends B1 and B3.

Green blend B2 had significantly lower ( $P < 0.05$ ) antioxidant activity than coffee blend B2 roasted at 167 and 171°C. Blends B1, B2 and B3 roasted at 167°C had significantly higher ( $P < 0.05$ ) antioxidant activity than these blends roasted at 175°C. According to results of some researches (Górnaś et al. 2016; Hečimović et al. 2011; Priftis et al. 2015; Sacchetti et al. 2009) the highest antioxidant activity was in the medium roasted coffee, while the dark roasted coffee usually had the lowest antioxidant activity.

According to the results of ABTS test, coffee blends B1 and B3 had the highest antioxidant activity at 167°C, significantly higher ( $P < 0.05$ ) than the same blends of green and roasted at 171 and 175°C. Blend B2 had significantly higher ( $P < 0.05$ ) antioxidant activity at 171°C compared to green blend B2 and roasted at 167 and 175°C. The lowest antioxidant activity was in green coffee blends B1, B2 and B3, significantly lower ( $P < 0.05$ ) than antioxidant activity in blends B2 and B3 roasted at 167, 171 and 175°C, and significantly lower ( $P < 0.05$ ) than antioxidant activity in blend B1 roasted at 167 and 171°C. Coffee blends B1 and B3 roasted at 171°C had significantly higher ( $P < 0.05$ ) antioxidant activity than these blends roasted at 175°C. Antioxidant activity was significantly higher ( $P < 0.05$ ) in blend B2 roasted at 167°C compared to antioxidant activity in blend B2 roasted at 175°C. Hečimović et al. (2011) found out that roasted coffee samples (Arabica and Robusta) had higher antioxidant activity (ABTS) than green coffee samples. Priftis et al. (2015) found out that Arabica coffee roasted at 215°C for 4 different roasting times had higher antioxidant activity (DPPH, ABTS) than green Arabica coffee.

Correlations between content of total phenol, total flavonoid and flavonol and antioxidant activity were calculated (Table 3). Significant correlations ( $P < 0.05$ ) were evident between TPC (mg CGA/g) and antioxidant activity (DPPH) and between FC (mg Q/g) and antioxidant activity (ABTS) of green coffee blends. A relatively good but not significant correlation ( $P > 0.05$ ) was found between TFC and the antioxidative activity of

**Table 3.** Correlations between content of total phenol, total flavonoid and flavonol and antioxidant activity of green coffee blends and coffee blends roasted at 167, 171 and 175°C

Green coffee blends (0°C)	DPPH ( $\mu\text{mol TE/g}$ )	ABTS ( $\mu\text{mol TE/g}$ )
TPC (mg (CGA)/g)	0,633P < 0,05*	-0,201P > 0,05* (ns)
TPC (mg (GAE)/g)	0,380P > 0,05* (ns)	-0,121P > 0,05* (ns)
TFC (mg (Q)/g)	0,497P > 0,05* (ns)	-0,571P > 0,05* (ns)
FC (mg (Q)/g)	0,388P > 0,05* (ns)	-0,693P < 0,05*
Roasted coffee blends (167°C)	DPPH ( $\mu\text{mol TE/g}$ )	ABTS ( $\mu\text{mol TE/g}$ )
TPC (mg (CGA)/g)	0,739P < 0,05*	0,842P < 0,05*
TPC (mg (GAE)/g)	0,283P > 0,05* (ns)	0,479P > 0,05* (ns)
TFC (mg (Q)/g)	0,484P > 0,05* (ns)	0,291P > 0,05* (ns)
FC (mg (Q)/g)	0,537P > 0,05* (ns)	0,268P > 0,05* (ns)
Roasted coffee blends (171°C)	DPPH ( $\mu\text{mol TE/g}$ )	ABTS ( $\mu\text{mol TE/g}$ )
TPC (mg (CGA)/g)	0,710P < 0,05*	0,612P < 0,05*
TPC (mg (GAE)/g)	0,956P < 0,05*	0,720P < 0,05*
TFC (mg (Q)/g)	0,180P > 0,05* (ns)	0,454P > 0,05* (ns)
FC (mg (Q)/g)	-0,253P > 0,05* (ns)	0,028P > 0,05* (ns)
Roasted coffee blends (175°C)	DPPH ( $\mu\text{mol TE/g}$ )	ABTS ( $\mu\text{mol TE/g}$ )
TPC (mg (CGA)/g)	-0,166P > 0,05* (ns)	-0,321P > 0,05* (ns)
TPC (mg (GAE)/g)	0,087P > 0,05* (ns)	0,150P > 0,05* (ns)
TFC (mg (Q)/g)	0,235P > 0,05* (ns)	0,034P > 0,05* (ns)
FC (mg (Q)/g)	-0,028P > 0,05* (ns)	0,371P > 0,05* (ns)

TPC – Total phenol content. TFC – Total flavonoid content. FC – Flavonol content. DPPH – 2,2-diphenyl-1-picrylhydrazyl. ABTS – 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid).

\*Pearson linear correlation coefficients. ns – not significant

these blends. Significant correlations ( $P < 0.05$ ) were evident between TPC (mg CGA/g) and antioxidant activity (DPPH and ABTS) of coffee blends roasted at 167°C. [Trandafir et al. \(2013\)](#) found a good correlation between total phenolics content of commercial coffee samples and their antioxidant activity. A relatively good but not significant correlation ( $P > 0.05$ ) was found between TFC and FC and the antioxidative activity (DPPH) of these blends. Correlations between TPC (mg CGA/g and mg GAE/g) of coffee blends roasted at 171°C and antioxidant activity determined with DPPH and ABTS were significant ( $P < 0.05$ ). These blends had the highest antioxidant activity. In correlations between content of total flavonoid and flavonol and antioxidant activity, Pearson linear correlation coefficients were not statistically significant ( $P > 0.05$ ). Correlations between content of total phenol, total flavonoid and flavonol and antioxidant activity of coffee blends roasted at 175°C weren't statistically significant ( $P > 0.05$ ). Based on these results it could be concluded that other coffee compounds, in addition to examined phenolic compounds such as chlorogenic acid, are also responsible for the antioxidant activity of coffee blends depending on the roasting degree.

#### 4. CONCLUSION

Results obtained in this study showed that coffee blend B3 roasted at 171°C had the highest antioxidant activity according to the DPPH test (240,63  $\mu\text{mol TE/g}$ ) and coffee blend B2 also roasted at 171°C had the highest antioxidant activity according to the ABTS test (240,81  $\mu\text{mol TE/g}$ ). Higher content of bioactive components TPC, TFC and FC was determined also in coffee blends B2 and B3. Those coffee blends had higher content of Robusta coffee than coffee blend B1 which had the highest content of Arabica 1<sup>st</sup> class. It can be concluded that medium roasting degree provides the highest antioxidant activity in coffee blends roasted in industrial conditions. Also, the higher content of Robusta coffee in the coffee blends contributes to a higher antioxidant activity of those blends. On the other hand, different ratios of two classes Arabica coffee in coffee blends have no significant effect on the increase of antioxidant activity of coffee blends roasted in industrial conditions. Results of this research can provide opportunities for the development of roasted ground coffee with higher antioxidant activity and improved nutritive quality, which is in accordance with the increasing demands of consumers.



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