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Physicochemical and sensory characteristics of green tea extract fortified steamed bread during storage

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Abstract

Complex physicochemical changes occur during the storage of steamed bread that are responsible for the firming and staling phenomena. In this research, instrumental and sensory analyses were conducted to investigate the effect of green tea extract (GTE) on the physicochemical and sensory characteristics of steamed bread before and after storage. Fortification of GTE as well as the storage conditions had no effect on the moisture content of steamed bread. GTE fortification of 0.25% and 0.50% resulted in similar specific volumes and crumb firmness compared to those of control, while 0.75% GTE significantly reduced the specific volume and increased the crumb firmness. After one week of refrigerated storage, the resteamed samples of bread containing 0.25% and 0.5% GTE were softer than the control. Differential scanning calorimetry (DSC) was used to determine the extent of amylopectin recrystallization during storage, and GTE showed a reducing effect on starch retrogradation. Sensory analysis by a trained panel indicated no difference in hardness, cohesiveness and stickiness by the fortifications of 0% (i.e. control), 0.25%, 0.50% and 0.75% GTE. While, increasing the GTE concentration led to an increase of astringency and a decrease of brightness.

Keywords steamed bread; green tea extract; sensory analysis; instrumental analysis; bread staling

1. Introduction

Steamed bread is commonly consumed as a staple food especially in northern China, and has gained increasing popularity in other parts of Asia. With the increasing demand for functional foods as consumers nowadays are realising that food and health are directly related, there has been a considerable interest in the possibility of incorporating health-promoting bioactives such as green tea into various traditional food items. Green tea polyphenols have demonstrated antioxidant, anti-inflammatory, antimutagenic and other beneficial properties that contribute to a lowered risk of chronic diseases and an improved state of health [1]. Green tea has been fortified in cereals, bread, cakes, biscuits, dairy products, meat, confectionary and oils [2,3], as a nutritional enhancer, flavour improver or a preservative, and also for preventing firmness in rice starch products [4]. Fortification of steamed bread with green tea extract (GTE) can enhance its nutritional value, making it a functional food that helps in disease prevention and health enhancement beyond the basic nutrition.

The quality of steamed bread is determined by its processing performance, volume growth, external characteristics, internal characteristics, eating quality and flavour. Good external characteristics include a smooth and shiny surface, symmetrical shape, large volume and white or bright colour [5,6]. A simple objective method has been developed focusing on colour and texture of steamed bread, that have an advantage in reproducibility over sensory methods of evaluating quality [7]. Four textural properties (softness, non-stickiness, elasticity and cohesiveness) and four external properties (skin colour, crumb colour, shininess and smoothness of the skin) have been chosen for the subjective evaluation of steamed bread, and it was found that there were significant correlations between instrumental and sensory measurements [7]. In addition, the quality of bread is also characterized by its moisture content, bulk density and porosity, while rheological properties of dough can be related to bakery products' specific volume and textural attributes.

The shelf life of steamed bread is relatively short due to its high moisture content, which is only 1 – 3 days (stored at room temperature) [8]. During storage, a series of physicochemical alterations take place, which contribute to the staling of steamed bre ad. As a consequence, the rigidity of the steamed bread increases, crumb resilience decreases and water evaporates from the surface, resulting in a firmer and drier feeling crumb [9]. It has been reported that the retrogradation of starch, specifically that of the short amylopectin side chains, are responsible for the bread firming during storage. Retrogradation of starch is the formation of crystalline state from an initially amorphous state that occurs in gelatinized starch [10]. Amylopectin crystallization plays a major role in the retrogradation of gelatinized starch due to their responsibility for long-term rheological changes, while amylose contributes to short-term changes. Water content and storage temperature influence the rate of retrogradation. Retrogradation is also responsible for reduced acceptability of many starch-containing foods [11].

Sensory analysis is a technique to analyse the food product's quality by using human responses as perceived through five senses, i.e. sight, smell, touch, taste and hearing [12]. Quantitative descriptive analysis (QDA) is a widely used method which involves the selection of panellists to confirm their ability to differentiate the sensory properties of food product to be tested [13]. This research aimed to investigate the effect of GTE on the sensory characteristics of steamed bread and its physicochemical properties during storage. Correlation between the sensory and instrumental analyses was conducted. The effect of GTE on starch retrogradation in the bread during storage was also assessed.

2. Materials and Methods

2.1. Materials

Steamed bread flour (proteins content 7.9%) and instant dried yeast (*Saccharomyces cerevisiae*) were obtained from Gim Hin Lee Pte Ltd (Singapore). Fine sugar and salt were purchased from NTUC Fairprice (Singapore). Green tea extract (GTE) was obtained from Pure Herbal Remedies Pte Ltd (Singapore), which was made from green tea (*Camellia sinensis*) leaves harvested in Guanxi, China. The GTE used in this study contained at least 65% of total tea catechins and 35% of epigallocatechins gallate (EGCG).

2.2. Steamed bread preparation

The materials used in making steamed bread dough were 1 kg of flour, 550 g water and 10 g each of dried instant yeast, salt and sugar. GTE was added at the levels of 2.5 g, 5.0 g and 7.5 g per kg of flour to produce steamed bread with 0.25%, 0.50% and 0.75% GTE, respectively. All ingredients were mixed using a spiral mixer for 4 min at a low speed of 100 rpm. The dough was then left at room temperature (22 ^{o}C) for 10 min. After that, the dough was flattened manually, and then divided into spherical pieces automatically by a moulder (DR. ROBOT[2], Daub Bakery Machinery B.V., Netherlands). The dough pieces were proofed in an incubator (Binder KBF 04-590, Binder Inc., Bohemia, NY, USA) for 45 min at 40 ^{o}C and 85% of relative humidity, and then were steamed in a stainless steel steamer (EGS63SSC, Singmah Steel Refrigeration, Singapore) for 20 min. Steamed bread samples were stored in polyethylene bags at room temperature (22 ^{o}C) for 1 day or at 4 ^{o}C in a refrigerator for 1 week, and resteamed for 20 min prior to analysis. Fresh and resteamed samples were left to cool at room temperature for 15 min before analysis.

2.3. Colour intensity and specific volume measurements

Colour intensity of steamed bread (skin surface) was measured using a spectrophotometer (Minolta CM 3500d, Japan) and indicated as the *L**, *a** and *b** values. The specific volume of whole steamed bread samples was measured using a Volscan Profiler (VSP 600, Stable Micro System Ltd, Surrey, U.K.).

2.4. Texture profile analysis (TPA)

TPA was carried out on control and GTE fortified steamed bread using a texture analyzer (TA.XT2i, Stable Micro system, Surrey, UK). Steamed bread samples were first halved vertically, and then the top cut off, producing samples with a thickness of 1.75±0.10 cm. The TPA test conditions were as follows: probe of 20 mm in diameter, pre-test speed of 1 mm/s, test speed of 2 mm/s, a 5 sec break between the first and the second compression, a load cell of 5 kg and a compression distance of 7 mm, i.e. 40% of sample thickness.

2.5. Moisture content analysis

The moisture content of fresh and stored samples of steamed bread before resteaming was determined using an oven method. Thin slices of the crumb were weighed (~2g) into an aluminium dish, and dried for 24 hours in a drying oven set at 105 ^{o}C. The samples were then weighed again after drying, and weight loss was attributed to moisture loss and thus moisture content of the samples.

2.6. Starch retrogradation analysis

Starch retrogradation measurements of stored steamed bread were carried out using a differential scanning calorimeter (Mettler-Toledo DSC822e, Switzerland) equipped with liquid nitrogen cooling accessories. Dry nitrogen gas was used to purge the surroundings of the furnace and furnace chamber at 200 ml/min and 80 ml/min respectively to prevent the condensation of water. Steamed bread crumb of 5-15 mg was weighed into a 40 µl aluminium standard pan (Mettler-Toledo, Switzerland) and hermetically sealed with an aluminium standard lid (Mettler-Toledo, Switzerland). The samples were heated from 25 ^{o}C to 90 ^{o}C at a rate of 5 ^{o}C/min, with an empty pan as a reference, and the enthalpy of retrogradation was evaluated from the endothermic peak in the thermogram, and calculated as J/g sample using the STARe software (version 8.01, Mettler-Toledo, Switzerland).

2.7. Sensory analysis

Sensory evaluation was performed following the combination method between the quantitative descriptive analysis and the spectrum method [14]. The assessment was conducted on three textural properties (hardness, stickiness and cohesiveness), and two surface properties (skin smoothness and brightness) as well as astringency of steamed bread. The attributes were judged in the following order: skin smoothness, hardness, cohesiveness, stickiness, astringency and brightness. Red light conditions were used throughout except for brightness to prevent colour bias, and plain water was provided for rinsing after each sample. Plain white bread was also provided to remove any residual astringent sensation during the evaluation of astringency. For each attribute, 3 samples were evaluated at once, and triplicate sessions were done.

2.8. Statistical analysis

All experiments were conducted at least in triplicates and results were reported as the mean values of the replicates and their standard deviations. Instrumental results were analyzed by single factor ANOVA at 0.05 significance level using SPSS 20 (IBM Corporation, New York).

3. Results and Discussion

3.1. Colour intensity

Colour is one of the important attributes that affect the acceptability of steamed bread [5]. Steamed bread originally has a white, bright and glossy colour. Fortification of GTE in the steamed bread changed its colour to a range of light brown to suntan brown depending on the GTE concentration added. Table 1 presents the colour intensity of steamed bread in terms of L^*, a^* and b^* values for the fresh ones, those after storage for one day at room temperature and those after storage for one week at refrigeration temperature. The results indicated that the brightness was reduced and the colour became darker with the fortification of GTE. Comparing the fresh and stored samples, there were no significant changes in the colour of steamed bread after storage.

Table 1. Colour intensity of steamed bread under various storage conditions

Samples	Intensity	0% GTE	0.25% GTE	0.5% GTE	0.75% GTE
Fresh	L^*	88.85 ± 0.72^a	76.04 ± 0.61^b	72.06 ± 0.59^c	68.49 ± 0.68^d
	a^*	0.44 ± 0.14^a	4.77 ± 0.49^b	7.48 ± 0.56^c	9.49 ± 0.48^d
	b^*	17.40 ± 0.71^a	17.36 ± 0.80^a	19.91 ± 0.70^b	21.75 ± 0.44^b
1 day	L^*	88.37 ± 0.46^a	75.45 ± 0.56^b	71.54 ± 0.57^c	68.39 ± 0.40^d
	a^*	0.37 ± 0.13^a	4.70 ± 0.28^b	7.45 ± 0.45^c	9.07 ± 0.25^d
	b^*	16.88 ± 0.62^a	16.70 ± 0.45^a	19.66 ± 0.68^b	21.41 ± 0.31^b
1 week	L^*	87.93 ± 0.45^a	75.14 ± 0.74^b	71.50 ± 0.44^c	68.55 ± 0.27^d
	a^*	0.31 ± 0.20^a	4.75 ± 0.35^b	7.74 ± 0.49^c	9.04 ± 0.22^d
	b^*	16.65 ± 0.75^a	16.36 ± 0.67^a	19.94 ± 0.63^b	21.32 ± 0.64^b

Means with the same superscript letters in each row are not significantly different ($p>0.05$)

Steamed bread without GTE addition had the highest L^* value and the lowest a^* as well as b^* values, which is considered having a brighter and whiter colour. Fortification of GTE resulted in lower L^* values and higher a^* and b^* values. These values implied a darker colour compiled with a reddish and yellowish appearance. The b^* values were similar between the control and bread with 0.25% GTE, and higher for those with 0.5% GTE and 0.75% GTE. Besides the contributing factor of the original brown colour of the GTE used, the darkening and increased redness could also be due to oxidation of catechins during the steaming process. Catechins can be oxidized to coloured compounds such as theaflavines and thearugubins, which contribute to the orange red and brown tones of tea liquors [15].

3.2. Specific volume

For baked bread, the formation of a porous structure and increasing of bread volume after baking depend on the ability of wheat gluten protein to retain the gas produced by yeast fermentation and oven spring in the early stage of baking [16]. Wheat flour dough has a unique viscoelastic characteristic that allows gas bubbles to expand and retain more gases; hence an expanded volume of bread can be produced, for both baked and steamed ones. The volume of GTE fortified steamed bread was influenced by the gas cell structure and gas retention ability of the dough during proofing and steaming. Fortification of 0.25% and 0.50% GTE did not have an adverse effect on the specific volume of steamed bread compared to that of the control, but the specific volume dropped significantly when 0.75% GTE was added as shown in Figure 1. The specific volume of steamed bread remained constant during both the room temperature and refrigerated storage, and resteaming also had no impact on the volume.

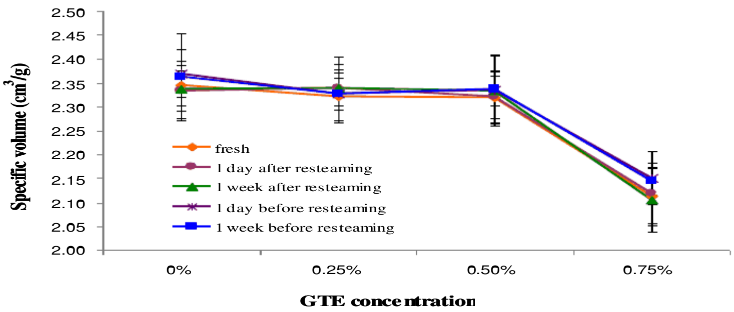

Figure 1. Specific volume of steamed bread under different storage conditions, with and without resteaming.

3.3. Texture properties

Texture plays an essential role in determining the quality of foods. Hardness is the main parameter in texture which shows the highest energy imparted in the first sample compression cycle of TPA. Hardness can also be used to define the power needed to break or destroy samples between the molar teeth. Cohesiveness is defined as a sample's internal strength that constructs the sample's structure [17]. Fortification of GTE affected the crumb hardness and cohesiveness of fresh steamed bread, as well as those before and after storage and resteaming.

The results of texture analysis showed that the fortification of GTE up to 0.50% had no impact on the crumb hardness of fresh steamed bread, but the fortification of 0.75% GTE resulted in higher crumb hardness than the other samples (Figure 2). After storage for one day at room temperature or one week at refrigeration temperature, the crumb hardness increased significantly by around 4 and 7 times respectively. However, the hardness was reduced significantly after resteaming, but was still higher than that of the fresh steamed bread. After a week's refrigerated storage, resteamed samples of bread with 0.25% and 0.5% GTE were softer than the control, which was possibly caused by the effect of GTE on retarding starch retrogradation. Fortification of 0.75% GTE contributed to higher crumb hardness, which could be due to its lower specific volume compared to the other samples. Bread staling and firming also contributed to the increasing of crumb hardness during storage.

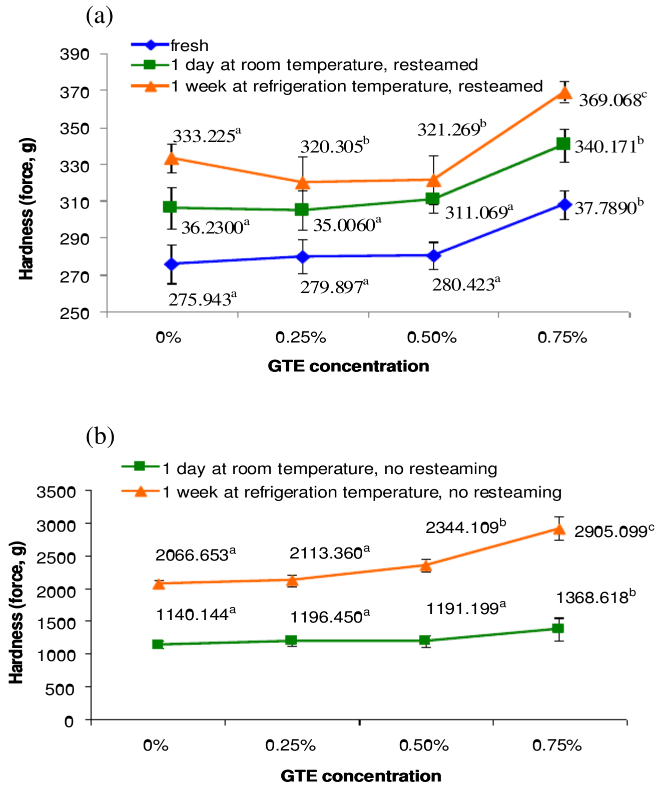

Figure 2. Change in hardness of steamed bread: (a) fresh samples and stored samples after resteaming, (b) stored samples before resteaming. Means with the same superscript letters are not significantly different (p>0.05).

3.4. Moisture content and starch retrogradation

Steamed bread is a high moisture product as steaming creates an environment that is saturated with water vapour, which could promote the absorption of water and prevent water loss that happens to baked bread. The moisture contents of the control and the GTE fortified steamed bread were approximately 43% (Table 2). The addition of GTE did not have any effect on the moisture content. During both the room temperature storage and the refrigerated storage, the moisture content of all samples remained constant and there was no water loss being observed. GTE did not seem to have any adverse effect on the moisture content during storage.

Table 2. Moisture content (% w/w, wet basis) of steamed bread under different storage conditions, without resteaming

GTE concentration	Fresh	1 day	1 week
0%	43.35 ± 0.50[a]	43.32 ± 0.57[a]	43.53 ± 0.48[a]
0.25%	43.03 ± 0.36[a]	43.08 ± 0.49[a]	43.25 ± 0.42[a]
0.5%	43.34 ± 0.37[a]	43.18 ± 0.45[a]	43.56 ± 0.51[a]
0.75%	43.04 ± 0.25[a]	43.21 ± 0.38[a]	43.35 ± 0.43[a]

Means with the same superscript letters in each column are not significantly different (p>0.05)

DSC analysis showed that GTE fortification reduced the extent of starch retrogradation. It was possibly due to the hydrogen bonding between tea polyphenols and starch. GTE addition decreased the reassociation of amylopectin chains into more crystalline organization. Table 3 shows the effect of GTE addition on the extent of starch retrogradation during the storage of steamed bread. After one day of storage at room temperature, there was no significant difference in starch retrogradation enthalpy between the control and the GTE fortified steamed bread. The effect of GTE on starch retrogradation was not obvious after such a short storage period. Starch retrogradation increased when steamed bread was stored for a week under refrigerated condition compared to a day at room temperature, not only due to the longer time for starch recrystallization to occur, but also due to the lower temperature which accelerated crystal formation. After a week's storage under refrigerated condition, the steamed bread containing GTE had significantly lower retrogradation endotherms compared to the control, which indicated that GTE retarded starch retrogradation.

Table 3. Retrogradation endotherms (J/g) of steamed bread crumb under different storage conditions

GTE concentration	1 day	1 week
0%	0.73 ± 0.11[a]	3.27 ± 0.47[a]
0.25%	0.82 ± 0.09[a]	2.70 ± 0.29[b]
0.5%	0.80 ± 0.14[a]	2.37 ± 0.20[b]
0.75%	0.79 ± 0.11[a]	1.89 ± 0.27[c]

Means with the same superscript letters in each column are not significantly different (p>0.05)

The retardation effect of GTE on starch retrogradation could be due to the presence of numerous reactive –OH groups in tea polyphenols. Tea polyphenols could compete to form hydrogen bonds with starch chains, thus preventing the alignment and aggregation of starch polymer chains, which would reduce starch retrogradation [4].

3.5. Sensory characteristics

Figure 3 shows that the panellists' perception of skin smoothness for the bread samples with 0.75% GTE appeared lower than those with the other levels of GTE; however the difference for the fresh steamed bread could not be observed statistically. The hardness, cohesiveness and stickiness were not significantly different among all the samples under the same storage condition. The instrumental analysis results showed that the fortification of 0.75% GTE produced higher crumb hardness, while the sensory scores of hardness were not significantly different among all the samples. In fact, the hardness became higher as a consequence of lower specific volume. However, in the sensory analysis, this increase could not be detected by the trained panellists.

Astringency is a tactile sensation that is usually described as a puckering, drying or rough mouthfeel, and is commonly associated with flavanols and their ability to precipitate proteins [18]. Tea catechins are able to precipitate salivary proteins, resulting in a loss in oral lubrication and therefore a drying sensation. As the concentration of GTE in the steamed bread increased, the perception of astringency generally increased. The astringency increased significantly at GTE fortification of 0.75% (Figure 3). It is interesting to note that astringency was also detected in the control samples, and this could be due to that plain water was used as the anchor reference for astringency at 1 in the 9–point scale.

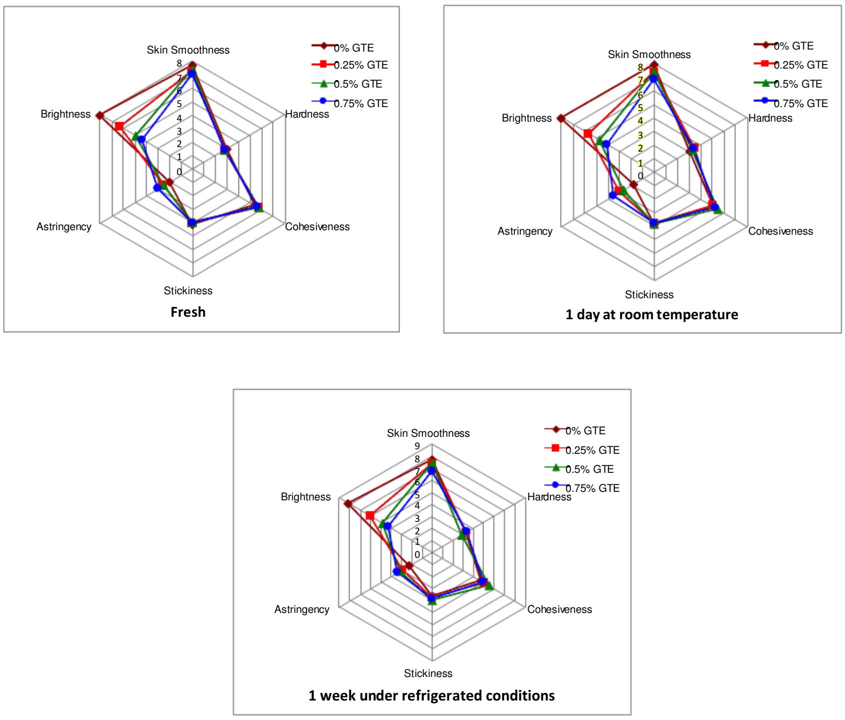

Figure 3. Sensory evaluation scores for different attributes and storage conditions.

3.6. Correlation between sensory evaluation and instrumental analysis

The sensory results on the brightness of steamed bread were highly linearly correlated with the *L** values obtained from using a spectrophotometer, with the coefficient of determination (R^2) being 0.97 and the root mean square error (RMSE) being 0.25 for the brightness. However, no correlation could be found between the sensory results and the texture analysis of steamed bread for the hardness and cohesiveness. The trained panellists could not detect any difference in the firmness and cohesiveness among all the samples.

Firmness is an important criterion to evaluate the quality of steamed bread. There was a very strong linear correlation between the instrumental crumb firmness and specific volume of steamed bread, with a R^2 value of 0.99 and RMSE of 0.65 for the crumb firmness. This correlation implied that a higher volume of steamed bread led to a decrease in the crumb firmness.

There was an inverse relationship between the amylopectin retrogradation and the instrumental crumb firmness of steamed bread upon storage, although the correlation was not very strong with a R^2 value of 0.82 and RMSE of 142.59 for the crumb firmness. Although it has commonly been accepted that amylopectin retrogradation contributes to and can be correlated with staling, the retrogradation is not solely responsible for the staling. As many mechanisms are associated with crumb firming and staling, the addition of GTE further complicated mechanisms involved, which may account for the poor correlation as there might be interactions between different factors.

4. Conclusion

GTE fortification at the highest concentration of 0.75% adversely affected the specific volume and crumb firmness of steamed bread. During storage, 0.75% GTE samples were consistently harder before and after resteaming for both the one day storage at room temperature and the one week storage under refrigerated condition. The addition of GTE had a significant impact on starch retrogradation, and a lower extent of retrogradation was observed with increasing GTE concentration after a week's storage. The starch retrogradation however did not correlate strongly with the crumb firmness during storage, despite an inverse relationship was shown.

Sensory analysis of steamed bread showed that the sensory threshold value for fortification of GTE into steamed bread in terms of hardness had not been reached yet as the panellists were unable to tell that the samples containing 0.75% GTE were harder than the others. However, the astringency of steamed bread was easily picked up at 0.75% fortification of GTE. The cohesiveness of steamed bread did not change with the addition of GTE, which was evident from both the sensory analysis and the texture profile analysis results.

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