Research Article

Assessment of physico-chemical and phyto-chemical properties of six apple varities cultivated in district Nagar and Hunza Gilgit Baltistan, Pakistan

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Abstract
This research work was carried out to evaluate the physico-chemical properties of six apple varieties commonly found in the local market of district Nagar and Hunza Gilgit, Pakistan. Different chemical and phytochemicals parameters such as moisture content, ash content, total soluble solids, pH value, acidity, total sugar, reducing sugar, non-reducing sugar, antioxidant activity and total phenolic contents were studied. Results revealed that highest moisture content was observed in Golden Delicious Hard (84.96%) while lowest moisture content was observed in Red Delicious (78.19%). Maximum ash content was found in Golden Delicious (2.92%) while minimum ash content was observed in Spartan (0.35%). Similarly, maximum mean value for total soluble solids were noted in Golden delicious (13.00) while minimum mean value was noted in Kala Kulu (10.56). Highest acidity was found in Golden Delicious (0.26%) whereas lowest acidity value was noticed in FS (0.16%). Maximum mean values for pH were recorded in Spartan (3.89) whereas minimum value was observed in Noor Shah (3.50). Total sugars content in apple varieties ranged from 66.78 to 73.66%. Maximum mean value for reducing sugars was noted in Noor Shah (14.54) while minimum value was recorded in Spartan (10.64). Non reducing sugar in apple varieties ranged from 51.54 to 54.00%. Highest antioxidant activity was recorded in Noor Shah (15.12%) while lowest value was observed in Spartan (18.61%). Total phenolic contents were ranged from 151.27 to 203.03 GAE/100mg. On the bases of physico-chemical properties it was concluded that red delicious variety is the best cultivar as compared to other varieties grown in this region.
Keywords: Apple varieties; Antioxidant potential; Gilgit Baltistan; Nagar-Hunza; Nutritional attributes

Introduction
Apple (*Malus pumila* L.) is a sweet, edible fruit, which is grown worldwide, and is the most widely cultivated in temperate zone. It belongs to *Rosaceae* family and is one of the most important perennial long-lived woody fruit crops of the world [1]. Due to high storage shelf life apple is available throughout the year and consumed in every season. Apple is mostly consumed as fresh and it ripens from late summer to winter. Apple is processed into many different

products which include jam, juices, puree, vinegar, jelly, frozen slices, canned slices and nectars [2]. Apple has good qualities it has to correspond to meet requirements of consumers and give satisfaction, needs, preferences, taste and habits [3]. Apple is one of the most important fruit species grown almost all over the world. According to the USDA, world total production of apples was 83.1 million tons. China is the topmost producer of apple producing 44 million tons annually which is 50% of world total production (USDA, 2018). According to the Pakistan Bureau of Statistics (2012/13); apples were produced over an area of 110,000 hectares with a total production of 556,000 metric tons, placing Pakistan on 11th position among the top 25 producers in the world.

Apple has great nutritional value and contains many nutrients including vitamin C, potassium and fiber. It also contains essential food constituents such as sugar (11%), proteins (0.3%), nearly 14% of apple is made up of carbohydrates, 4% minerals and remaining part contains 80% of water [4]. Different research revealed that apple fruit contained sufficient minerals that include Ca, K, Mg, Na, P and Zn. The Ca levels of apple fruits ranged between 7215.2 mg/kg to 10116.2 mg/kg [5]. Apple fruit has many health benefits which help in the maintenance of healthy life. The health benefits are associated with prevention from cancer, cardiovascular diseases, asthma, diabetes, Alzheimer's and diabetes. Red delicious apple has strong antioxidant activity, it contain quercetin which help in strengthen immune system of human body [6]. It was reported that apple have strong nutraceutical properties, so it was considered as defined functional food [7].

Apples are generally considered as the "sweet gold" of Pakistan and are among the most popular fruits. In Pakistan, it is grown as a commercial crop in Punjab, Khyber Pakhtunkhwa, Quetta and Gilgit Baltistan (GB) [8]. In Nagar and Hunza are famous for producing good quality apples.

According to the Statistics Department of GB; apple cultivation area is about 3 thousand hectares where annually production is about 10 thousand metric tons. Apple is one of the commonly grown cash crops in Gilgit-Baltistan [9] and has a significantly impact on income generation of poor people of mountain communities [10]. It is fourth important cash crop in the world after grapes, citrus fruits and banana [11].

Nagar and Hunza are blessed with many natural resources including fruits among which apple is the most famous. In Nagar and Hunza districts; different apple varieties are grown which include Red delicious (RD), Golden delicious (GD), Golden delicious hard, Kala kulu, Spartan and Noor shah. Different apple varieties are found in this area but due to lack of interest and awareness some varieties of apple are near to extinction and their chemical composition are still not explored [12].

The main purpose of this research work was to evaluated physico-chemical characterization of six apple varieties usually grown in Hunza Nagar to identify the best apple cultivar among six apple varieties on the basis of physical and chemical properties. Present study helped to explore the nutritional and phytochemical composition of different apple varieties grown in Hunza Nagar district.

Materials and methods

The research was conducted in the Department of Food Technology, Institute of Food and Nutrition Sciences, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan. Six verities of apple were collected from Research farms of study area at commercial maturity stage and transported to the laboratory. Fruits were cleaned and washed to remove all foreign agents i.e. dust particles, dirt, and sand and sorted to separate damaged and unripe fruits.

Chemical parameters

Moisture Content

The moisture of fruit sample was determined by AOAC method No.934-06 [13]. The following formula was used to calculate the moisture contents:

$$\text{Moisture} = \frac{\text{Wt. of fresh sample (g)} - \text{Wt. of sample after drying(g)}}{\text{Wt. of sample (g)}} \times 100$$

Total soluble solids

Total soluble solid of fruit of sample was determined by using refractometer, as described by AOAC method No.920-151 [13].

Total sugars

Total sugar content of apple varities were determined by Lane and Etymon method described in AOAC method No.925-35 [13].

Reducing sugars

Reducing Sugar contents of fruit sample was determined by Lane and Etymon method as described in AOAC method No. 925-36 [13].

Non-reducing sugars

Non-reducing Sugar content of was determined by subtracting total sugar from reducing sugar.

$$\text{Non} - \text{reducing Sugars} = \text{Total Sugars} - \text{Reducing Sugars}$$

Ash contents

The ash content was determined by incinerating the dried sample in muffle furnace at 500-600°C for 5 to 6 hours as described in AOAC method No.940-26 [13].

pH

The pH value of fruit sample was measured by using pH meter as described in AOAC method No.918-12 [13].

Titratable acidity

Titratable acidity in term of malic acid (eq. wt. 67.05) of apple fruit samples was determined by using AOAC method No.981-12 [13].

Antioxidant activity

Antioxidant activity of fruit samples was determined by using modified version of Brand-William [14]. It involved the use of free radical 1.1-diphenyl-2-picrylhydrazy (DPPH).

Total phenolics (mg GAE/100g)

The total phenolic content of fruit sample was determined by using the Foilin-Ciocalteau,s as described by Sponas and Wrolstad [15].

Physical parameters

Weight (W)

Weight of apple cultivars was determined by a digital electronic balance with 0.001g sensitivity, selected fruit randomly from each variety.

Length (L)

Lengths of fruits were determined by digital caliper (0-150 mm, China) with an accuracy of 0.001 mm.

Width (W)

Widths of all apple verities were measured by digital caliper (0-150 mm, China) with an accuracy of 0.001 mm.

Thickness (T)

Thicknesses of fruits were measured by digital caliper (0-150 mm, China) with an accuracy of 0.001 mm.

Volume (V)

Volume of apple samples was measured by the liquid displacement method.

Take a measuring jar and filled with water, note the level of water. Put an apple in measuring jar and record the level of water after putting apple in jar. The final volume of apple was calculated by using formula [16].

Volume =Final reading-Initial reading

Diameter

The Geometric mean diameter (D_g) was calculate by using given equation

$D_g = (L+W+T)^{0.333}$

Where L is length, W is width and T is thickness of the fruit [17].

Surface area (S)

Surface area of different apple varieties were determined according to method of Mosheim [17] by using following formula:

$S = \pi D_g^2$

Where D_g is the Geometric mean diameter of the fruit.

Statistical analysis

The data was analyzed and interpreted by using analysis of variance technique, comparison of means and other relevant statistical tools [18].

Results and discussion

Different laboratory test were carried out determined the physico-chemical composition of all six apple varieties taken from study area. Chemical parameters involved; moisture contents, ash contents, total soluble solids, ph, titratable acidity total sugars, reducing sugars and non-reducing sugars. phytochemical experiments involved antioxidant activity and total phenolics contents. physical parameters; weight, length, width, thickness, volume, diameter and surface area. The data is recorded in (Table 1 to 5) and statistically interpreted by 1 way ANOVA using statistical software (Minitab and SPSS). The results were discussed as follow.

Chemical parameters

Moisture content (%)

The moisture content of fruits and vegetable is an important indicator for determining the keeping quality and also an important sign of freshness of any food commodity. The moisture content of different apple varieties ranged from 78% to 88%, (Table 1). Highest moisture content was observed in Noor Shah that contained 87.55% while the lowest moisture content was recorded in Red Delicious apple variety that was 78.19% as shown in (Table 1). This work was correlated with previous research of Campeanu et al. [19], Chakespari et al. [20] and Kheiralipour et al. [21] indicated that moisture level in apple fruit varies from 77-88%. This study results were matched with previous finding.

Ash contents (%)

The ash content is a measure of the total amount of minerals present within a fruit. Mean result showed that ash content of different apple varieties ranged from 0.35 to 2.92% and the high value was recorded in Red Delicious (2.92%) while low level of ash was recorded in Spartan (0.35%) as shown in (Table 1). Our finding was well supported by previous worked of Campeanu et al. [19] and Mukhtar et al. [22] carried for ash analysis of apple, suggested that ash contents in different apple verities are ranged from 0.28 % to 2.98% The outcomes of our this studied showed resemblance with previous research.

pH

It is evident from statistical result that the values was found to be significant (P<0.05) among apple verities. The mean values for pH were fall in the range of 3.50 to 3.92. The result revealed that highest level of pH recorded was 3.92 (Red Delicious), while lower level was 3.50 (Noor Shah) as shown in (Table 1).

Physicochemical analysis of different apple varieties was carried out by different scientists. Previous studied showed that pH values of different apple varieties ranging from 3.55 to 4.27 respectively Campeanu et al [19], Laplace et al. [23] and Vieira et al. [24]. The results of present research showed similarity with previous worked.

Titratable acidity (%)

The statistical results revealed that the Titratable acidity was found to be significant (P<0.05) among all verities. While analysis of total acidity of six apple cultivars, the following outcomes were recorded as; 0.26% in golden delicious, 0.16% in red delicious, 0.21% in Golden Delicious Hard, 0.24% in Kala kulu, 0.18% in Spartan and 0.28% in Noor Shah. The mean values showed that highest acidity present in golden delicious (0.26%) while lowest amount of acidity is 0.16% present in red delicious variety as shown in (Table 1).

Titratable acidity of fruit is an important parameter in determining fruit maturity and key determinant of fruit taste. It also serves as food substance and need by body in little amounts. Researchers showed that titratable acidity of different apple varieties

grown in world ranged from 0.10 % to 0.36% respectively Chakespari *et al.* [20], Vieira *et al.* [24] and Durrani *et al.* [25].

Current findings for titratable acidity in apple varieties interlinked with previous results.

Table 1. Mean moisture (%), ash (%), pH and titratable acidity (%) of six apple varieties

Apple varieties	Moisture (%)	Ash (%)	pH	Titratable acidity (%)
Golden Delicious	84.58±0.75AB	0.96±0.05C	3.76±0.30C	0.26A±0.03
Red Delicious	78.19±4.00C	2.92±0.13A	3.92±0.03A	0.16C±0.02
Golden Delicious Hard	84.96±1.14AB	0.68±0.05D	3.83±0.02AB	0.21AB±0.03
Kala Kulu	83.56±2.38B	1.18±0.06B	3.73±0.09C	0.24AB±0.03
Spartan	82.61±1.38B	0.35±0.04E	3.89±0.16AB	0.18C±0.03
Noor shah	87.55±1.65A	0.63±0.07D	3.50±0.06D	0.28A±0.04

The values are means of three replications with standard deviation (SD)

Total Soluble Solids (Brix°)

The statistical result showed that TSS was significantly (P<0.05). While analysis of total soluble solids (TSS) of six apple varieties by using refractometer the mean results result shoed that total soluble solid of apple varieties ranged from 10.56 ° to 13.00° which was presented in (Table 2). The result showed that high level of TSS 13.00° was observed in apple cultivar (Golden Delicious) while lowest level was indicated as 10.56 ° in Kala Kula variety. This findings were correlated with previous worked of Campeanu *et al.* [19], Chakespari *et al.* [20], Laplace *et al.* [23], Vieira *et al.* [24], Durrani *et al.* [25] and Muhammad *et al.* [26] suggested that total soluble solids contents in different apple verities grown in world ranging from 8.5° to 17° respectively. Our research outcomes showed conformance with previous studied.

Total Sugars (%)

Apples are rich source of sugars (reducing and non-reducing). The sugar profile is a significant constituent of chemical composition and provides important information concerning the authenticity of fruit juices. The analysis of variance for total sugars of apple varieties is given in Appendix VI. The statistical result revealed that the total sugars were differ significantly (P<0.05) among all verities. The mean value of total sugar level of six apple cultivars was in between 64.73% and 73.66% which were presented in (Table 2). It was clear from result that the highest of

total sugars were found in Golden Delicious which was 73.66% while Kala Kulu variety contained lowest value of total sugars which was 64.73%. Previous worked on physico-chemical parameter of fruits and showed that total sugars in apple varieties ranged from 51% to 80% Maqsood *et al.* [2] and Campeanu *et al.* [19] showed resemblance with current worked.

Reducing sugars (%)

Reducing Sugar of six apple verities was investigated in this study. It is cleared from the data that reducing sugars in apple cultivars was non-significant (P<0.05). The result indicated that reducing sugars of different apple verities ranged from 10.64% to 16.90% as mentioned in (Table 2). While analysis of reducing sugar it was observed that highest value found in Red delicious (16.90%) while low value was observed as 10.64% in Spartan variety.

This studied results were compared with different previous research of Vieira *et al.* [24] which investigated that reducing sugars in different apple varieties ranges from 3 % to 12% Current research result did not show resemblance with previous worked. It would be due to varietal factor.

Non-reducing sugars (%)

It is obvious from statistical result that non reducing sugars were differed significantly (P<0.05) among different cultivars. The mean results showed that non-reducing sugar of six apple verities were ranged from 51.54% to 60.59% which were indicated in (Table 2). The result

substantiated that high valve of non-reducing sugar was recorded in Golden delicious while low valve was observed in Kala kulu cultivar which were 60.59% and 51.54% respectively. Previous studies described that non reducing sugar in different apple varieties ranged from 48 % to 65% [1] showed similarity with present research.

Table 2. Mean TSS, total sugars (%), reducing sugars (%) and non-reducing sugars (%) of six apple varieties

Apple Varieties	TSS (º)	TS (%)	RS (%)	NRS (%)
Golden Delicious	13.00±0.20A	73.66±0.82A	13.71±1.38BC	60.59±1.00A
Red Delicious	11.40±0.30C	69.50±1.05B	16.90±1.11A	51.54±2.48C
Golden Delicious Hard	11.50±0.20B	69.57±1.30B	12.50±1.23CD	59.63±3.23A
Kala Kulu	10.56±0.35D	64.73±2.39C	15.73±0.98AB	51.54±1.50C
Spartan	11.20±0.20C	66.78±1.33C	10.64±0.51D	55.96±1.20B
Noor Shah	11.63±0.25BE	69.70±0.94B	14.54±2.04BC	54.00±1.11B

The values are means of three replications with standard deviation (SD)

Phyto-chemical parameters
Antioxidant activity (%)
Fruits rich in antioxidants help in lowering prevalence of degenerative disease like arteriosclerosis, cancer, cardiovascular [6]. Antioxidant is substance that can prevent or delay oxidative damage of protein, lipids and nucleic acids by reactive oxygen groups such as free radicals. They scavenge radicals by inhibiting and breaking chain or suppressing formation of free radicals by binding to metals ions, reducing hydrogen peroxide and quenching superoxide and singlet oxygen [27]. Data regarding means values of antioxidant showed that antioxidant activity was found in the range of 15.12% to 24.44% as mentioned in (Table 3). The means result of all verities showed that maximum antioxidant activity was recorded in Red Delicious (24.44%) and minimum antioxidant activity in Noor Shah Variety which was (15.12%).
Results were compared with Manzoor *et al.* [28] reported that antioxidant level in apple fruit ranges from 13% to 42%. Similarly Boyer & Liu [6] showed that apple fruit contain antioxidant level ranged from 10% to 36%. A difference in antioxidant activity was also observed among fruit cultivars. Apples showed antioxidant activity over a wide range, from 14.7% to 40.7% Karadeniz *et al.* [29].

Total phenolic contents (mg GAE/100g)
Phenolics are bioactive compound that plays an important role in plant defense mechanism and also in expression of antioxidants in plants. During analysis of total phenolivs contents the following results were drawn; 184.700 in golden delicious, 203.03 in red delicious, 173.90 in Red Delicious Hard, 189.76 in Kala Kulu, 151.27 in Spartan and 170.87 in Noor Shah. Mean results showed that higher phenolic contents were found in Red Delicious (203.03 mg GAE/100g) while Spartan variety contained lowest level of phenolics contents i.e. 151.27 mg GAE/100g as shown in (Table 3).
These results were correlated with the work of Boyer & Liu [6] which indicated that total phenolic level in different apple varietes are in between 115.0 mg GAE/100g to 225.5 mg GAE/100g. It was reported that total phenolics contents ranged from 110 to 357 mg/100g of fresh apple Liu *et al.* [30]. Current results showed similarity with previous worked.
Physical parameters
Physical characteristics of agricultural products are the most important parameters for determination of proper standards of design of grading, conveying, processing, and packaging systems. Fruit weight is important to determine the best harvesting

time and utilization of resources also depend on size and weight of fruit has significant influence in determine packaging and sizing system [30].

Table 3. Mean of antioxidant activity (%) and Total phenolics contents of six apple varieties

Varieties	DPPH (%)	Total Phenolics Contents (mg/GA)
Golden Delicious	21.40±0.09C	184.70±0.06C
Red Delicious	24.44±0.07A	203.03±0.11A
Golden Delicious Hard	20.90±0.05D	173.90±0.10D
Kala Kulu	22.66±0.06B	189.76±0.09B
Spartan	18.61±0.09E	151.27±0.12F
Noor Shah	15.12±0.63F	170.87±0.61E

The values are means of three replications with standard deviation (SD)

Weight (mg)

The statistical analysis concluded that the fruit weight among all apple varieties were differ significantly (P<0.05). The result showed that the apple fruits weight ranged from 106.46 mg to 235.36 mg and the highest value regarding weight was 235.36 and lowest value was 106.46 in Red delicious and Spartan varities respectively as given (Table 4) [31].

This worked was compared with previous worked of Kheiralipour et al. [21] and Tabatabaeefar & Rajabipour [32] indicated that different apple varities weight ranging from 51 g to 368 g and showed conformance with current finding.

Length (mm)

It was clear from the results the length of all apple varities ranged from 42.57 to 73.70mm. The result further investigated that the longest apple among all varieties is Red Delicious apple which contained 73.70 mm length while lowest value of length was 42.57 in Spartan variety as shown in (Table 4). The finding results was in line with the studied of Kheiralipour et al. [21], and Ozturk et al. [33] suggested that length of different apple and other fruits ranged from 40 mm to 80mm

Width (mm)

During analysis for width of different apple varities the results recorded as: 58.96mm in golden delicious, 74.99mm in red delicious, 59.68 in Red Delicious hard, 57.51 in Kala Kulu, 53.91 in Spartan and 52.21 in Noor Shah. The result showed that reading recorded in different varities were in between 52 mm to 75 mm. The highest value recorded was 74.99 mm in Red Delicous variety while the lowest value mention as 52.21 mm in Noor Shah apple variety as shown in (Table 4). It was suggaested that width of different apple varities ranged from 50mm to 110mm Kheiralipour et al. [21]. Current findings were interacted with early studied.

Thickness (mm)

The result showed that reading was recorded in different varities were ranging from 53 mm to 72 mm. The maximum thickness was recorded as 71.33mm in Red Delicious variety while the minimum thickness reported as 52.70mm in Spartan variety as mentioned in (Table 4). This studied was linked with the work of Kheiralipour et al. [21] who investigated that thickness of apple fruit ranged from 55mm to 95mm. Results are statistically significant (p<0.05).

Volume (mm)

Volumes and projected area of fruits must be known for accurate modeling of heat and mass transfer during cooling, drying, transporting, packing and storing. The mean values for fruit volume has been presented in (Table 5). The result concluded that values for volume of all varieties were ranging from 79mm to 107mm. The result further investigated that highest volume was recorded in Red Delicious apple (106.60) while lowest volume was found in Noor Shah variety (78.72). The result of this study showed conformity with the previous work of

Kheiralipour *et al.* [21] and Ozturk *et al.* [33] who suggested that volume of apple and pear ranged from 55mm to 170mm.

Table 4. Mean weight, length, width and thickness of six apple varieties

Varieties	Weight (mg)	Length (mm)	Width (mm)	Thickness (mm)
Golden Delicious	165.40C±3.19	58.15B±0.90	58.96C±3.06	54.24BC±2.10BC
Red Delicious	235.30±2.04A	73.70±3.60A	74.99 ±2.08A	71.33±3.50A
Golden Delicious Hard	189.30±1.46B	54.63±1.70C	59.68±2.17B	55.04±2.60BC
Kala Kulu	191.00±4.05C	56.82±1.07B	57.51±5.11C	56.22±4.29BC
Spartan	129.50±3.90D	42.57± 2.08 D	53.91±2.05D	52.70±1.10C
Noor Shah	106.40±4.45E	51.54± 2.20C	52.21±2.09D	57.78±1.88B

The values are means of three replications with standard deviation (SD)

Diameter (mm)

The mean values for fruit diameter have been presented in (Table 5). The results showed that mean values for diameter were ranging from 50 to 73 mm and table further investigated that the maximum volume was recorded in Red Delicious apple and minimum volume was shown in Dhnag variety which were 73.02mm and 49.69mm respectively. Current results were correlated with previous worked, studied on the role of physical and chemical performance during storage of apple cultivar, investigated that geometric diameter of different apple varieties recorded as 57mm to 95mm Kheiralipour *et al.* [21] and Banoo *et al.* [34] showed highly significant.

Surface area (mm)

Surface area would be useful in determination of mass of the cubicula membrane per unit fruit surface area [35]. While analysis of apple varieties for surface area it was concluded that surface area of all apple varities ranged from 7750.90 mm to 16803.5mm. The result further investigated that the highest surface area was noted in Red Delicious and lowest was recorded in Spartan variety that were 16803.5 mm and 7750.90 mm respectively as shown in (Table 5). This study was also correlated with the previous work of Kheiralipour *et al.* [21] who early worked of some physical properties two different verities.

Table 5. Mean volume, diameter and surface area of six apple varieties

Varieties	Volume(mm)	Diameter (mm)	Surface Area (mm)
Golden Delicious	98.56±1.15B	57.05±1.01B	10203.8±100.3B
Red Delicious	106.6±4.96A	73.02±2.94A	16803.50±102.5A
Golden Delicious Hard	88.59±3.29C	56.65±1.76B	9954.350±16.15C
Kala Kulu	104.70±1.22A	56.77±1.07B	10129.70±21.20B
Spartan	80.08±1.11D	49.69±1.20C	7750.90±12.10D
Noor Shah	78.72±1.58D		9916.30±68.00C

The values are means of three replications with standard deviation (SD)

Conclusion

The results of current research work revealed that apple varieties cultivated in Nagar and Hunza valleys have rich nutritional and phytochemical components such as total sugar, ash contents, antioxidant activity, and total phenolics. On the basis of physico-chemical properties it was concluded that Red Delicious variety is the best cultivar as compared to other varieties grown in this region. The current study provides first hand physico-chemical information of six apple cultivars that will be supportive for the researchers and growers in developing

postharvest management systems and industrialization of apple fruit in the area.

Author's contributions

Conceived and experiments designed: A Sohail, Performed the experiments: A Mehdi, M Mazahir & M Asim, Analyzed the data: KS Abbasi & M Mazahir: Contributed regeants/ materials/ analysis tools: KS Abbasi & RM Amir, Wrote the paper: A Mehdi, A Sohail & W Ali.

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