

The effects of different harvest periods to bio-active compounds in wheat

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ARTICLE INFO

Received: February 12, 2021

Received in revised form: December 1, 2021

Accepted: December 2, 2021

Keywords:

Antioxidant

Anthocyanin

Harvest

Phenolic compound

Wheat

ABSTRACT

Natural antioxidants, especially plant phenolics, such as anthocyanins, are reliable and have a history of food use; they are also bio-active so consumption of plant extracts from natural sources is increasing day by day. The aim of this study is to detect the effects of different harvest periods on some growth parameters and bio-active compounds in wheat. The study was conducted in the 2015-2016 growing season in Konya. Seeds of Bezostaja 1, AN 110 and AT 053 genotypes were used. Growth parameters and bio-active compounds were determined on the grains of spike samples obtained at 6 different harvest periods. Whilst all of the three genotypes of the trial had the highest TA (Total Anthocyanin) levels at 1st harvest; genotype AN 110 had the highest TAnt. (Total Antioxidant) content at 1st, and Bezostaja 1 and AT 053 had the highest TAnt. levels at the 6th sampling. Bezostaja 1 had the highest TPC (Total Phenolic Content) at 1st sampling; AN 110 had the highest TPC at 3rd and AT 053 at the 4th harvest period. Total antioxidant values decreased until the 3rd sampling then increased at the 4th, 5th and 6th harvest periods. Whilst the values of GM (Grain Moisture), SFW (Spike Fresh Weight), TA and TPC traits decreased on going maturity stages; values of GDW (Grain Dry Weight), SDW (Spike Dry Weight) and TGW (Thousand Grain Weight) features increased linearly. According to the results some of the growth parameters and bio-active compounds of wheat grains that were harvested earlier were higher.

1. Introduction

Wheat is one of the main compounds of human nutrition especially in developing countries. Wheat varieties have some characteristic properties that determine their usage purposes. For example if the gluten content of wheat in hard endosperm is higher, that allows its use in making bread and cakes. In wheat, quality is related to grain gluten content, grain colour and nutrition. Colour is one of the important quality traits in pasta production and results of genotypic factors most of the time (Adom et al. 2003; Marconi and Carcea 2001; Hentschel et al. 2002). Anthocyanins are chemical molecules that are responsible for blue, purple, red and orange colours in plants (Havrlentova et al. 2014). Anthocyanins are compounds that have high antioxidant activities, anti-inflammatory and many positive effects on human health. They are bio-active compounds that are found in high levels in fruits, vegetables and cereals and have many health benefits (Abdel Aal et al. 2018; Shipp and Abdel-Aal 2010). Wheat grain and its derivatives include phytochemicals that have high antioxidant activities such as phenolic acids, carotenoids and tocopherols (Yu et al. 2013). Wheat antioxidants are also important in bread making. Nowadays people prefer abstaining from white flour for a healthier lifestyle. It is known that consuming whole wheat grain effects human health positively because of the synergetic effects of phytochemicals (Arshad et al. 2017). Natural antioxidants are consumed more than artificial ones because of their positive effects on physiological systems. Antioxidants neutralize free radicals, detoxify ROS (reactive oxygen species), act as metal chelators and inhibit oxidative enzymes. Reactive

oxygen species trigger uncontrolled cell development and cause DNA damage (Harris and Kris-Etherton 2010; Arshad et al. 2017; Flight and Clifton 2006). Cereals have come to the attention of researchers with their phenolic compounds that have strong antioxidant effects and health benefits. Concentrations of phenolic compounds in cereals depend on the variety and the parts of the grain. Phenolic compounds are generally classified as phenolic acids, flavonoids, stilbenes, coumarins, lignans and tannins. These compounds exist in the grain as sugar derivatives and form complexes with organic acids, amines, lipids and other phenols (Liu 2007; Zilic et al. 2011; Zilic 2016). Nowadays the positive effects of bio-active compounds on human health has encouraged producers to develop varieties with higher antioxidants and proteins. The suitability of bio-active compounds of wheat is affected by treatments before harvest, milling and storage conditions (Cheng et al. 2006; Arshad et al. 2017). Harvesting time also effects bio-active compounds (Merendino et al. 2006; Paradiso et al. 2006). Changes of bio-active compounds according to different harvest periods were investigated in this study.

2. Materials and Methods

2.1. Materials

In the trial Bezostaja 1 patented wheat variety and two wheat lines that were developed by Prof. Dr. Ali TOPAL from

SUAF (Selcuk University Agriculture Faculty) Crop Science Department were used.

2.2. Methods

The study was conducted in the 2015–2016 growing seasons in Konya/Turkey (32°, 31' N, 37°, 52' E) at SUAF Crop Science Department, Prof. Dr. Abdulkadir AKÇIN trial area. The trial was established according to “Factorial Experimental Design in Randomised Blocks” with three replications. Seeds were sown in the winter in parcels that have 4 rows; each was 2 m long. Distance between two rows was 20 cm. There were 500 seeds in each square meter. Irrigation cycles were completed during the sowing, stem elongation and spike formation stages respectively. Fertilisation was given according to the following calculation; 6 kg da⁻¹ P₂O₅ (sowing), 10 kg da⁻¹ N (1/3 in sowing, 2/3 in spring). Spike formation data was recorded during spike formation at 50% of each parcel (first week of May). Six samplings (6th June, 13th June, 20th June, 28th June, 4th July, 11th July in 2016) were done at different harvest periods at one week intervals. The relative humidity value of June was 48.2% and 41.6% in July in 2016 (MGM 2017). Eleven spike samples were taken from each parcel at each sampling time. These spikes were transferred to the laboratory immediately in plastic bags. The following parameters were detected in spike and grain samples.

GFW (Grain Fresh Weight): Grains were separated from fresh spikes and weighed. The mean of eleven values was recorded as gram “GFW”.

GDW (Grain Dry Weight): Spikes were left at room conditions for 7 days then oven dried at 35°C for 48 hours. Grains were separated from spikes and were weighed. The mean of eleven values was recorded as gram “GDW”.

SFW (Spike Fresh Weight): Spike samples were put in plastic bags and moved to the laboratory immediately. Spike samples were weighed with precision scales. The mean of eleven values was recorded as gram “SFW”.

SDW (Spike Dry Weight): Fresh spike samples were left at room conditions for 7 days then oven dried at 35°C for 48 hours. Spike samples were weighed with precision scales; values were recorded as gram “SDW”.

GM (Grain Moisture): Grains that were separated from fresh spikes were weighed then oven dried and re-weighed. Values obtained from these stages were subtracted. Moisture values were recorded as per cent “GM”.

TGW (Thousand Grain Weight): Dry grains in each spike were counted and weighed. Means were calculated. Values were used by the following formulae and recorded as gram “TGW”.

$$TGW (g) = (1000 \times \text{Grain Weight per Spike}) / \text{Grain Number per Spike}$$

TA (Total Anthocyanin): Total anthocyanin analysis was performed according to Leticia et al. (2009). 0.1 g grain sample was homogenised with 5 ml propanol:HCl:water solution. The extract was boiled after centrifuge and left at room conditions for 24 hours. After the last centrifuge ABSs of the supernatants at 535–650 nm were detected with a spectrophotometer.

TAnt. (Total Antioxidants): Grain total antioxidant activity was determined according to Khampas et al. (2013) by the spectrophotometric method. 0.5 ml phenolic extract was homogenised with 5 ml DPPH (60mM) solution. Supernatants

were left at room conditions for 30 minutes after vortex; then ABS values were detected at 517 nm by spectrophotometer.

Values were obtained with the following formulae and recorded as % “TAnt.”

$$\text{Scavenging rate (\%)} = [(A_0 - A_1) / A_0] * 100$$

TPC (Total Phenolic Content): Total phenolic content of grains was detected according to Ma et al. (2016). 2.0 g grain sample was homogenised in 16 ml methanol including 1% HCl. Homogenate was centrifuged and supernatant was stored at +4°C. 5 ml Folin–Ciocalteu solution was added to 0.5 ml phenolic extract and the solution was neutralized with 4 ml sodium carbonate (75 g l⁻¹) than left for two hours at room conditions. Absorbances of the supernatants were determined at 765 nm with a spectrophotometer.

2.3. Statistical analysis

All data shown are the mean values (n=3). Data were statistically analysed by the analysis of variance (ANOVA) with MSTAT-C software using Duncan’s multiple range test at the level of significance $P < 0.05$.

3. Results

In the trial GDW, GFW, GM, SDW, SFW, TA, TAnt. and TPC traits of cultivar Bezostaja 1 and lines AN 110, AT 053 were reviewed at six different harvest periods. Variance analysis results of each trait and mean values are presented in Table 1 and Table 2 respectively. In the trial, while values of GFW, GM, SFW, TA and TPC features decreased, compared to previous samplings; GDW, TGW and SDW properties increased linearly. Fluctuations were observed in TAnt. among sampling times (Table 2, Table 3). Whilst Bezostaja 1 variety was investigated according to GDW values; the highest GDW levels were observed at the 4th and 5th sampling times in accordance with what had been expected. Grain dry weight values increased linearly, until the 5th sampling. In the last sampling time, GDW value decreased by 13% compared to the previous one. Grain dry weight value of line AN 110 increased at the 2th sampling; decreased by 33% at the 3rd sampling compared to the previous one and increased by 34% at the 4th sampling compared to the 3rd one as well. A linear increase was observed in variety AT 053 compared to two other genotypes in GDW. A 3% decrease was observed at the 5th sampling and a 5% increase was observed at the next sampling time. Line AT 053 had the highest GDW value at the final sampling. Although TGW values of Bezostaja 1 increased linearly up to the 4th sampling, values decreased in the 5th and 6th sampling periods (Table 2). The TGW values of Bezostaja 1 decreased by 14.06% and became 51.53 g then moisture loss continued and grain weight became 49.32 g at the 6th sampling. Thousand grain weight values of line AN 110 increased linearly up to the 6th harvesting time and then decreased by 12.21% compared to the previous one. A similar situation was observed in variety AT 053 as well; TGW value decreased by 7.39% compared to the previous sampling time (Table 2). Spike dry weight value of line AN 110 was 3.910 g at the 5th sampling and became 2.800 g at the last sampling by losing weight of 28.38%. Increasing SFW values of each genotype at the first time stopped by the 4th, 5th and 6th samplings. Spike fresh weight value (3.490 g) of variety Bezostaja 1, decreased by 34% at the 5th sampling. Similarly increasing SFW value till the 4th sampling of

Table 1. Variance analysis results of each trait

Source of Variation	DF	GDW (g)	GFW (g)	GM (%)	TGW (g)	SDW (g)	SFW (g)	TA (mg kg ⁻¹ C3G)	TAnt. (%)	TPC (mg kg ⁻¹ GAE)
Replication	2	0.000	0.001	7.436	18.134	0.284	0.443	1.124	0.000	31.818
Genotype	2	0.020**	0.029**	229.243**	1634.616**	4.869**	21.670**	18.480**	168.061**	10916.514**
Error	4	0.000	0.002	11.255	43.952	0.060	0.036	0.789	0.921	4.477
Sampling Time	5	0.001**	0.075**	3744.715**	2662.561**	3.073**	6.200**	619.243**	233.283**	79142.211**
Genotype * Sampling Time	10	0.002	0.005	64.142**	61.536**	0.253**	0.842**	33.053**	99.330**	60787.355**
Error	30	0.037	0.016	13.863	20.336	0.032	0.156	0.295	2.114	143.308
Total	53
CV (%)		8.88	12.95	9.24	11.07	7.74	9.70	6.27	4.99	1.48

** $P < 0.01$

DF (Degree of Freedom), CV (Coefficient of Variation), GDW (Grain Dry Weight), GFW (Grain Fresh Weight), GM (Grain Moisture), TGW (Thousand Grain Weight), SDW (Spike Dry Weight), SFW (Spike Fresh Weight), TA (Total Anthocyanin), TAnt. (Total Antioxidant), TPC (Total Phenolic Content).

Table 2. Mean values of each genotype obtained from different sampling times

Genotypes	ST	GDW (g)	GFW (g)	GM (%)	TGW (g)	SDW (g)	SFW (g)	TA (mg kg ⁻¹ C3G)	TAnt. (%)	TPC (mg kg ⁻¹ GAE)
Bezostaja	1	0.193 fg	0.563 cde	65.74 a	17.44 i	0.820 h	2.360 i	20.540 b	26.70 fg	322.3 a
	2	0.240 ef	0.573 bcd	57.25 bcd	29.06 h	1.530 g	3.590 fg	8.260 e	24.30 gh	183.0 b
	3	0.337 bcd	0.633 abc	47.09 ef	45.10 efg	1.880 f	3.560 fg	4.650 gh	18.37 i	122.5 i
	4	0.369 abcd	0.623 abc	37.38 g	60.22 bc	2.000 ef	3.490 gh	3.840 hi	23.45 h	130.7 h
	5	0.369 abcd	0.359 f	12.04 i	51.53 de	2.070 ef	2.280 i	5.510 fg	27.15 f	122.8 i
	6	0.318 d	0.420 f	11.26 i	49.32 def	2.160 ef	2.430 i	2.760 j	38.14 bc	113.8 j
AN 110	1	0.164 g	0.429 f	62.01 ab	6.25 j	1.580 g	4.160 def	32.850 a	44.78 a	147.0 f
	2	0.248 ef	0.599 abc	58.89 bc	17.66 i	2.240 e	5.430 b	10.850 d	26.14 fg	140.1 g
	3	0.227 ef	0.461 def	48.22 ef	27.31 h	2.780 cd	6.110 a	5.430 fg	24.87 fgh	186.4 b
	4	0.344 abcd	0.629 abc	45.48 f	39.65 g	3.330 b	6.090 a	2.770 j	22.75 h	144.9 f
	5	0.339 bcd	0.453 ef	26.23 h	49.32 def	3.910 a	5.330 bc	1.020 k	22.65 h	112.8 j
	6	0.325 cd	0.439 f	25.56 h	43.29 fg	2.800 cd	3.750 efg	4.710 gh	30.51 e	55.88 l
053	1	0.257 e	0.702 a	63.03 ab	19.67 i	1.550 g	4.190 def	21.030 b	31.50 e	172.0 c
	2	0.313 d	0.685 ab	54.73 cd	29.55 h	1.950 ef	4.300 de	11.870 c	26.28 fg	161.5 d
	3	0.318 d	0.660 abc	51.71 de	53.35 cd	2.770 cd	5.770 ab	5.510 fg	26.28 fg	151.2 e
	4	0.389 ab	0.578 bcd	37.79 g	62.87 ab	2.920 c	4.690 cd	5.820 f	34.20 d	173.2 c
	5	0.376 abc	0.419 f	10.66 i	68.27 a	2.580 d	2.880 hi	3.610 ij	36.45 cd	111.4 j
	6	0.397 a	0.443 f	10.05 i	63.22 ab	2.600 d	2.890 hi	5.010 fg	39.70 b	104.4 k
LSD (0.05)		0.052	0.117	6.209	7.520	0.298	0.658	0.905	2.424	3.645

GDW (Grain Dry Weight), GFW (Grain Fresh Weight), GM (Grain Moisture), TGW (Thousand Grain Weight), SDW (Spike Dry Weight), SFW (Spike Fresh Weight), TA (Total Anthocyanin), TAnt. (Total Antioxidant), TPC (Total Phenolic Content).

Table 3. Mean values of each traits at each sampling time

Sampling Time	GDW (g)	GFW (g)	GM (%)	TGW (g)	SDW (g)	SFW (g)	TA (mg kg ⁻¹ C3G)	TAnt. (%)	TPC (mg kg ⁻¹ GAE)
06.06.16	0.204 c	0.565 a	63.589 a	14.448 d	1.310 d	3.562 c	24.800 a	34.321 b	213.774 a
13.06.16	0.267 b	0.619 a	56.950 b	25.417 c	1.900 c	4.433 b	10.320 b	25.563 d	161.516 b
20.06.16	0.294 b	0.585 a	49.000 c	41.912 b	2.471 b	5.140 a	5.190 c	23.164 e	153.343 c
28.06.16	0.367 a	0.610 a	40.209 c	54.239 a	2.744 a	4.751 b	4.137 d	26.793 d	149.577 d
04.07.16	0.359 a	0.432 b	16.303 d	56.366 a	2.844 a	3.017 d	3.374 e	28.742 c	115.656 e
11.07.16	0.347 a	0.414 b	15.617 d	51.937 a	2.510 b	3.490 c	4.151 d	36.111 a	91.354 f

GDW (Grain Dry Weight), GFW (Grain Fresh Weight), GM (Grain Moisture), TGW (Thousand Grain Weight), SDW (Spike Dry Weight), SFW (Spike Fresh Weight), TA (Total Anthocyanin), TAnt. (Total Antioxidant), TPC (Total Phenolic Content).

variety AN 110 decreased at 5th sampling time (Table 2). A similar situation was observed for variety AT 053 as well (Table 2). While Bezostaja 1 investigated according to TA levels it was observed that the highest TA was obtained from materials of the first sampling time. The most prominent decrease was observed at the second harvesting time. The total anthocyanin content of grains obtained during the second sampling, decreased 58.78%

compared to the previous sampling time. Decrease of TA levels of variety Bezostaja 1 continued linearly at all other harvesting times (Table 2). The highest TA level of AN 110 was obtained from the first harvesting time, similar to Bezostaja 1 (Table 2). The total anthocyanin level of line AN 110, decreased by 60% at the second harvesting period. This critical decline continued at other harvesting times as well. In addition the TA level of AN

110 increased to 78% at the last harvesting time. While TA values obtained from line AT 053 were investigated it was observed that the highest TA was detected at the first sampling, similar to the other two genotypes. Total anthocyanin level (21.03 mg kg⁻¹ C3G) of line AT 053 decreased linearly at sampling times (Table 2). At the last sampling time the TA level increased by 29.94% as line AN 110. A wide variation was also observed for TAnt. levels of all genotypes of the trial. While a linear decrease was observed at the first three sampling times of variety Bezostaja 1, a 21% increase was observed at the 4th sampling time and this situation continued until the last harvesting time. The highest antioxidant values were obtained from the last harvesting time. Total antioxidant content of line AN 110 decreased linearly up to the last harvesting time; a 25.76% increase was observed at the last harvesting time compared to the previous one. Whilst line AT 053 was investigated according to TAnt. content, a 16.57% decrease was observed at the second sampling. Changes of the TAnt. content were not observed at the 3rd sampling. A 23.15% increase was observed at the 4th sampling period compared to the previous one; this situation continued linearly up to the last sampling time. Line AT 053 had the highest antioxidant level at the last sampling time. While genotypes of the trial were investigated according to TPC levels, it was observed that variety Bezostaja 1 had the highest TPC at the first sampling time. Total phenolic content decreased up to the 4th sampling. A little increase (6.27%) was observed at the 4th sampling, then the decrease continued up to the last sampling. Decreasing TPC of line AN 110 at the 2nd sampling time; a 24.83% increase was observed compared to the previous one at the 3rd sampling period. Total phenolic content that started decreasing during previous samplings became the lowest at the last sampling. Fluctuations were observed in TPC feature of line AT 053 as well. Total phenolic content of grain samples decreased up to the 4th sampling. An increase of 30.05% was observed at the 4th sampling, the decrease continued at two other sampling times. The lowest TPC was detected at the last sampling period (Table 2).

4. Discussion

Phenolics are subunits with high biological activities in cereals; consumption of these antioxidants decreases risk of cardiovascular diseases and some types of cancer. Nowadays including high radical scavenging antioxidants, increases the popularity of coloured wheat varieties (Lutsey et al. 2007). Anthocyanins are water soluble natural colourants that belong to the flavonoid class of phenolic phytochemicals (Liu 2004). In the trial it was observed that TA and TPC levels of wheat grains decrease parallel to each other at later maturity stages (Table 3)

supported by previous literature a significant and positive relation was observed between TA and TPC traits as well (Table 4). Zofajova et al. (2012) determined TA levels changed between 2.37 and 291.07 mg kg⁻¹ C3G; in the same study genotype ANK 28 had the highest anthocyanin level at the 3rd maturity stage and it had no anthocyanins at the 6th one. In this research a significant and negative correlation was observed between TA and SDW features (Table 4). The decrease of anthocyanins' from water soluble flavonoids at the next stages of harvest were found to be significant and it is thought that the decrease of the anthocyanin level can be related to moisture loss. The positive relation between anthocyanin levels and grain properties in this study also supports this induction as well (Table 4). Kenievel et al. (2009) reported that starch content increased during the on-going harvest period. The speed of dry matter accumulation in endosperm is higher than dry matter accumulation in *alueron* and *pericarp* thus accumulation speeds of anthocyanins in endosperm become slower. Wheat grains and fractions have high antioxidant activities, phenolic compounds, many phytochemicals, carotenoids and tocopherols. Phytochemicals and antioxidants in wheat support the immune system and prevent many diseases. Consumption of whole wheat grain decrease the risk of cardiovascular diseases and types of cancer (Arshad et al. 2017). Among the genotypes of the trial a wide variation was observed according to antioxidant contents (Table 2). Total antioxidant levels decreased up to the 4th sampling time, though increased in the next one. The increase of TAnt. level at the 4th maturity stage continued up to the next maturity stages. Saha et al. (2018) reported that they determined TAnt. levels of wheat extracts between 0.39%-80.10%. While maturity of bio-active compounds in wheat was investigated; it was observed that TA, TAnt. and TPC levels were higher at early maturity stages parallel with higher moisture levels. In the literature there are many studies whose results are also compatible with this study. De Gara et al. (2003) and Paradiso et al. (2006) reported that wheat grains with higher moisture content (70%) have higher antioxidant activities compared to wheat grains with lower moisture levels. It was declared that TAnt. levels were higher at early maturity stages and increase 2–3 weeks after flowering in the same study. Wheat is generally consumed as an energy source besides being rich in fibre, minerals, antioxidant compounds and bio-active phytochemicals. Phytochemicals are phenolic compounds and synthesis under stress conditions as secondary metabolites (Levakova and Bartoza 2017). Some phytochemicals are bound and cannot be digested by human enzymes. These kinds of compounds take part in the digestion process in colons through fermentation. This fermentation process occurs in colons

Table 4. Correlation coefficients of all traits with each other

	GDW	GFW	GM	TGW	SDW	SFW	TA	TAnt.
GFW	-0.44
GM	-0.83*	0.86**
TGW	0.98**	-0.51	-0.86**
SDW	0.96**	-0.39	-0.78*	0.98**
SFW	-0.05	0.79*	0.51	-0.07	0.06
TA	-0.40	0.32	0.72	-0.90**	-0.94**	-0.19
TAnt.	-0.12	-0.62	-0.30	-0.11	-0.30	-0.74*	0.35	...
TPC	-0.84*	0.72	0.93**	-0.85*	-0.80*	0.26	0.86**	-0.12

* $P < 0.05$, ** $P < 0.01$

and has many benefits to human health. In many epidemiologic studies researchers reported that consuming whole grains prevents chronic diseases such as colon cancer, gastro intestinal cancers and breast cancer (Liu 2007; Gabor et al. 2006; Narwal et al. 2014). According to the results of this study anthocyanin levels, obtained during earlier sampling stages, were higher and then decreased during the on-going harvest period. A similar situation was observed for total phenolics as well (Table 3). Ma et al. (2016) determined bounded TPC between 603.10 $\mu\text{g g}^{-1}$ -917.20 $\mu\text{g g}^{-1}$, and free TPC between 67.94 $\mu\text{g g}^{-1}$ -113.66 $\mu\text{g g}^{-1}$ in wheat samples. A significant and positive correlation was also observed between TAnt. and TPC traits in this study (Table 4). A lot of literature has recorded higher levels of TPC in grains at early maturity stages; TPC levels decrease at on-going maturity stages for wheat grain (McCallum and Walker 1990; Shao et al. 2014; Ma et al. 2016). Lewis et al. (1999) reported that sucrose is necessary for polyphenol biosynthesis; it is also necessary for starch synthesis. Starch bio-synthesis accelerates at early and mid-stages of grain filling. At these stages starch bio-synthesis is faster than polyphenol bio-synthesis so polyphenol synthesis decreases at on-going stages of grain filling periods in wheat (Ma et al. 2016). This situation causes the increase of the starch level and a decrease in anthocyanin contents. The findings of this study support this literature as well.

5. Conclusion

In this study it was observed that the antioxidant capacity of wheat grains were higher at earlier harvest periods. These results have highlighted the possibility of using early harvested wheat grains as a source of bio-active compounds. The findings may indicate that early selection can be applied for traits that do not have regular linear decreases in the negative direction, such as the TA character. The output of the current work indicates the necessity and/or possibility of developing/producing materials rich in these kinds of bio-active compounds.

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