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Antioxidant potential of kvasses

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This study was aimed at determination of antioxidant capacity of commercial kvasses. Four different beverages ("Obolon", "Wileński", "Gubernija", and Eko-Natura") were purchased in local shops in Poland, one beverage ("Brottrunk Biovegan") originated from the German market. Antioxidan capacity of the beverages was investigated using ABTS, FRAP, and DPPH assays. The content of total phenolic compounds was determined using a Folin-Ciocalteu's phenol reagent. The profile of phenolic compounds were determined using an HPLC method. The content of total phenolics ranged from 0.083 to 0.372 mg/ml; the TEAC values from 0.133 to 1.001 μmol Trolox/ml; the FRAP values from 0.893 to 3.079 μmol Fe^{2+}/ml. The antiradical activity against DPPH radical ranged from 0.097 to 0.463 μmol Trolox/ml. A strong correlation was noted between the contents of total phenolics and results of antioxidant assays. The presence of benzoic acid in one beverage was confirmed using the HPLC method.

Keywords: Fermentation; Beverages; Kvass; Antioxidant activity; Phenolic compounds, HPLC

INTRODUCTION

Fermentation has been exploited by the mankind for millennia to preserve and enhance food. Fermented foods and beverages have a healthy, natural, clean label and safe image, but current practices for many food fermentations (broad spectrum activities of natural fermentation) are generally based on traditional methods, where specific culture selection methodologies are applied with focus on techno-functional characteristics as opposed to bio-functional capacity of the microbiota used in the fermentation process.

In Central and East Europe very popular are fermented beverages called as kvass. Kvass is a non-alcoholic beverage produced by fermenting kvass mash with yeast; alcohol content in kvass must be less than 1.2% alcohol by volume [1-3]. In kvass investigated by Dluskaya *et al*. [4], *Lactobacillus casei* and *Leuconostoc mesenteroides* were present in cell counts of 7.3×10^7 and 6.0×10^7 CFU ml^{-1}, respectively. *Saccharomyces cerevisiae* was present in cell counts of 3.0×107 CFU ml^{-1}. Russian kvass was characterized by energy value of 4 kcal/100 g and contents of proteins and carbohydrates of 0.115 and 0.98 g/100 g, respectively [6]. The predominant carbohydrates of kvass are maltose, maltotriose, glucose, and fructose [6]. In kvass samples Lidums *et al.* [7] detected 25 volatile compounds. Ten of them were esters, five alcohols, five acids, four aldehydes, and three ketones.

Considering the antioxidant potential of cereals, we decided to analyze antioxidant capacity of commercial kvass from Polish and German markets as well as to determine the profile of phenolic compounds of kvass.

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EXPERIMENTAL

Chemicals

Methanol and acetonitryle were acquired from the P.O.Ch. Company (Gliwice, Poland). Ferrous chloride, sodium persulfate, the Folin-Ciocalteau phenol reagent, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), (+)-catechin, benzoic acid, *p*-coumaric acid, rutin 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tri(2-pyridyl)-s-triazine (TPTS), and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were obtained from Sigma (Poznań, Poland).

Material

Four different beverages ("Obolon", "Wileński", "Gubernija", and Eko-Natura") were purchased in local shops in Poland, one beverage ("Brottrunk Biovegan") was originated from the German market.

Total phenolics content

The content of total phenolics in the examined beverages was investigated using Folin and Ciocalteu's phenol reagent [8] using (+)-catechin as a standard.

ABTS assay

Antiradical activity against ABTS•+ was determined as Trolox equivalent antioxidant capacity (TEAC) and investigated according to Re *et al*. [8]. Results were expressed as μmol of Trolox per 1ml of beverage.

FRAP assay

Ferric reducing antioxidant power for tea samples was conducted according to method described by Benzie at al. [9]. Results were expressed as μmol of Fe^{2+} per 1ml of beverage.

DPPH assay

Antioxidant capacity against DPPH radical was tested using method described by Yen and Chen [11]. Results were expressed as μmol of Trolox per 1ml of beverage.

HPLC analysis

An HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of CTO-20AC column oven, two LC-30AD pumps, an CBM-20A system controller, SIL-30AC autosampler and an SPD-M30A photodiode array detector was used to separate the phenolic compounds in the kvass samples. A 10 μL of filtered sample was injected onto a Luna C18(2) column (150 × 4.6 mm, 3 μm, Phenomenex, Torrance, CA, USA). Gradient elution of acetonitrile-water- trifluoroacetic acid (5:95:0.1, v/v/v) [solvent A] and acetonitrile-trifluoroacetic acid (100:0.1, v/v/v) [solvent B] with a flow rate of 1 mL/min was used. Solvent B was increased from 0 to 60% from 0-18 min, decreased and maintained at 0 from 18.20-20.0 min [12]. The diode array detection was performed by scanning over a wavelength range of 200 to 400 nm. The wavelength of 280 nm was selected for quantification of the major phenolic constituents. The quantification was based on calibration curves of (+)-catechin and benzoic acid.

Statistical analysis

All analyses were triplicated. The results were reported a mean values± standard deviation. The linear correlation coefficients between the content of total phenolic compounds and the results of the antioxidant activities assays of beverages were calculated using Pearson's test. Principal component analysis (PCA) was also used. STATISCITA 10, StatSoft was used in this research for statistical analysis.

RESULTS AND DISCUSSION

The content of total phenolic compounds ranged from 0.086 (Obolon) to 0.372 μg/ml (Wileński) (Table 1). The highest antiradical activity determined using ABTS assay was obtained for Wileński (1.001 μmol Trolox/ml) and the lowest for Obolon (0.133 μmol Trolox/ml). Wileński was also characterized by the highest reducing (3.079 μmol Fe^{2+}/ml) and antiradical activity against DPPH radical (4.64 μmol Fe^{2+}/ml). The lowest results of FRAP and DPPH assays were was obtained for Obolon (0.893 μmol Fe^{2+}/ml) Eko-Natura (0.073 μmol Trolox/ml), respectively.

The content of total phenolics in kvasses was lower than that reported by Coda *et al*. [13] for emmer beverages fermented by selected lactic acid bacteria: 0.31- 0.95 mmol gallic acid equivalents/l). The content of total phenolic of fermented beverage based on germinated and ungerminated seeds of barley, finger millet ranged from 1.40 to 2.48 mmol gallic acid equivalents/l [14]. The low content of phenolic compounds in commercial kvass was probably caused by the application of filtration process which removed small particles of cereals from the beverages. It can be confirmed by the fact that all beverages were without any turbidity.

Table 1. Characteristic of investigated kvasses: content of total phenolics and results of ABTS, FRAP, and DPPH assays.

Beverage	Total phenolics (μg/ml)	ABTS assay (μmol Trolox/ml)	FRAP assay (μmol Fe^{2+}/ml)	DPPH assay (μmol Trolox/ml)
Obolon	0.086 ± 0.001	0.133 ± 0.002	0.893 ± 0.015	0.131 ± 0.008
Wileński	0.372 ± 0.007	1.001 ± 0.024	3.079 ± 0.063	0.464 ± 0.020
Gubernija	0.221 ± 0.010	0.654 ± 0.014	2.121 ± 0.042	0.215 ± 0.010
Eko-Natura	0.189 ± 0.005	0.470 ± 0.015	1.747 ± 0.028	0.073 ± 0.009
Brottrunk Biovegan	0.118 ± 0.003	0.351 ± 0.008	1.242 ± 0.028	0.089 ± 0.006

The antiradical activities against DPPH radical was reported before for rice [15], emmer [13] and barley, finger millet and moth bean [14] fermented beverages. The ability to scavenge ABTS radical cation by phenolic compounds of fermented beverage based on barley, finger millet and moth bean was reported by Chavan *et al*. [14].

The presence of several phenolic compounds in kvass samples was confirmed by HPLC (Figure 1, Table 2). The highest content was obtained for compound 5 in Wileński and Gubernija. Benzoic acid (typical synthetic antioxidant) was detected and determined in Obolon and Wileński (compound 9).

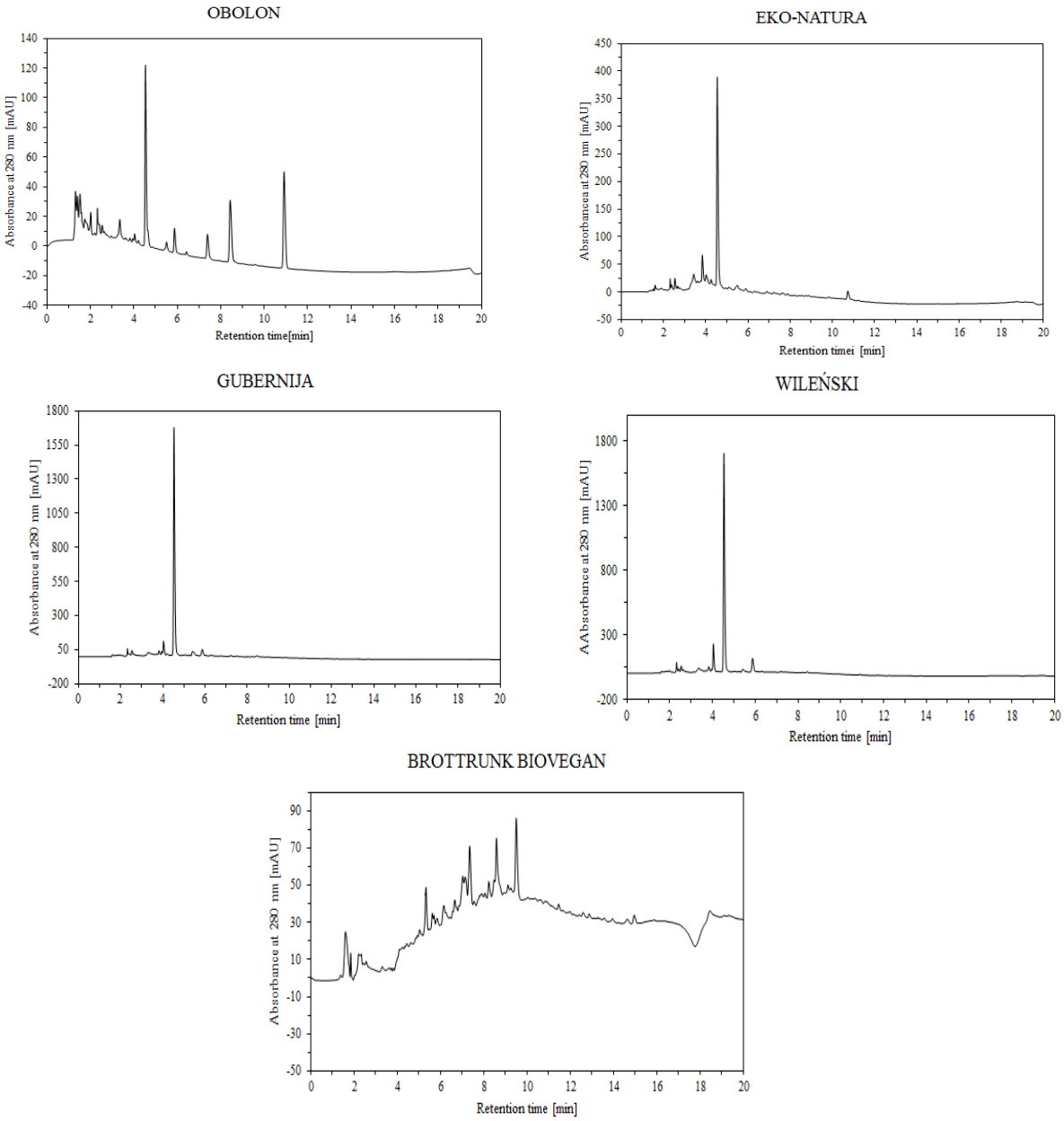

Fig. 1. HPLC chromatograms of phenolic compounds of kvass samples

Figure 2 depicts UV-DAD spectra of kvass samples. They were characterized by absorption maxima between 259 and 295 nm. The lack of spectra with maxima of absorption around 320 nm may indicate that ferulic acid and its derivatives were not extracted into water or were metabolized during fermentation. In this work, for the first time, a correlation was calculated between the content of phenolic compounds in kvass samples and their antioxidant activity (Figure 3). The correlation coefficients between the total phenolics content and the results of the ABTS and FRAP assays were 0.881 and 0.781, respectively.

A similar relationship between the content of total phenolics in leguminous and lupin extracts and their antioxidant activities was previously reported by Amarowicz *et al*. [16], Orac *et al.* [12, 17], and Karamać *et al*. [18]. For 24 different cereals, correlation coefficients between total phenolics in plant material and their antioxidant potential determined using ABTS and FRAP assays were 0.940 and 0.942, respectively [19].

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Table 2. Content of individual phenolic compounds (mg/ml) in kvasses

Compund (retention time)	Beverage				
	Obolon	Wileński	Gubernija	Eko-Natura	Brottrunk Biovegan
1 (2.32 min)	0.015 ± 0.001	0.065 ± 0.003	0.043 ± 0.002	0.011 ± 0.000	-
2 (2.55 min)	0.005 ± 0.000	0.053 ± 0.002	0.044 ± 0.002	0.018 ± 0.001	-
3 (3.85 min)	-	0.064 ± 0.003	0.027 ± 0.001	0.114 ± 0.005	-
4 (4.03 min)	0.004 ± 0.000	0.053 ± 0.003	0.098 ± 0.004	0.056 ± 0.002	-
5 (4.55 min)	0.146 ± 0.007	1.884 ± 0.091	1.854 ± 0.092	0.468 ± 0.020	-
6 (5.97 min)	0.021 ± 0.001	0.163 ± 0.008	0.073 ± 0.003	-	0.003 ± 0.000
7 (7.39 min)	0.025 ± 0.001	-	-	-	0.009 ± 0.000
8 (8.43 min)	0.075 ± 0.003	0.025 ± 0.001	0.016 ± 0.001	-	0.009 ± 0.000
9 (benzoic acid) (10.92 min)	0.109 ± 0.005	0.010 ± 0.001	-	-	-

Content of compounds 1-8 is expressed as catechin equivalents

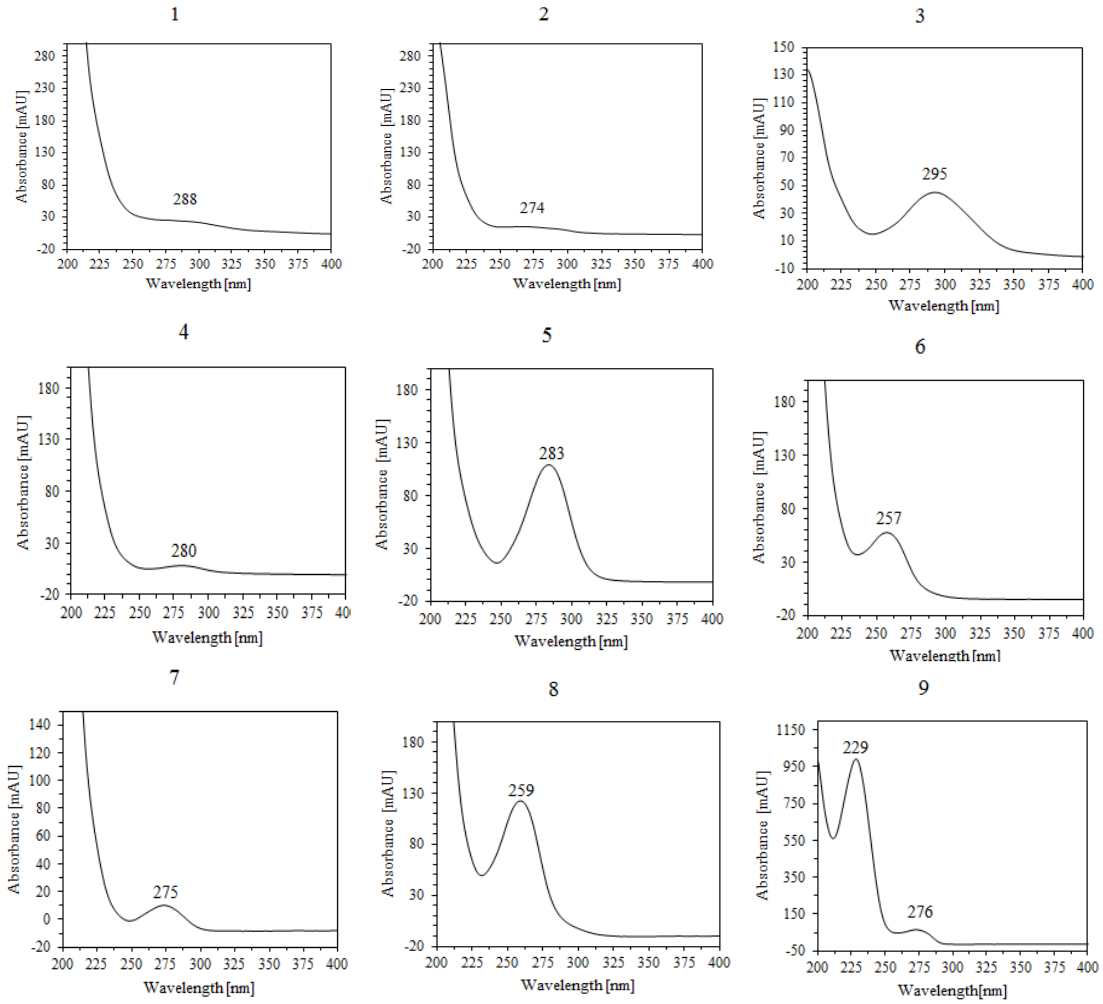

Fig. 2. UV-DAD spectra of individual phenolic compounds of kvass samples.

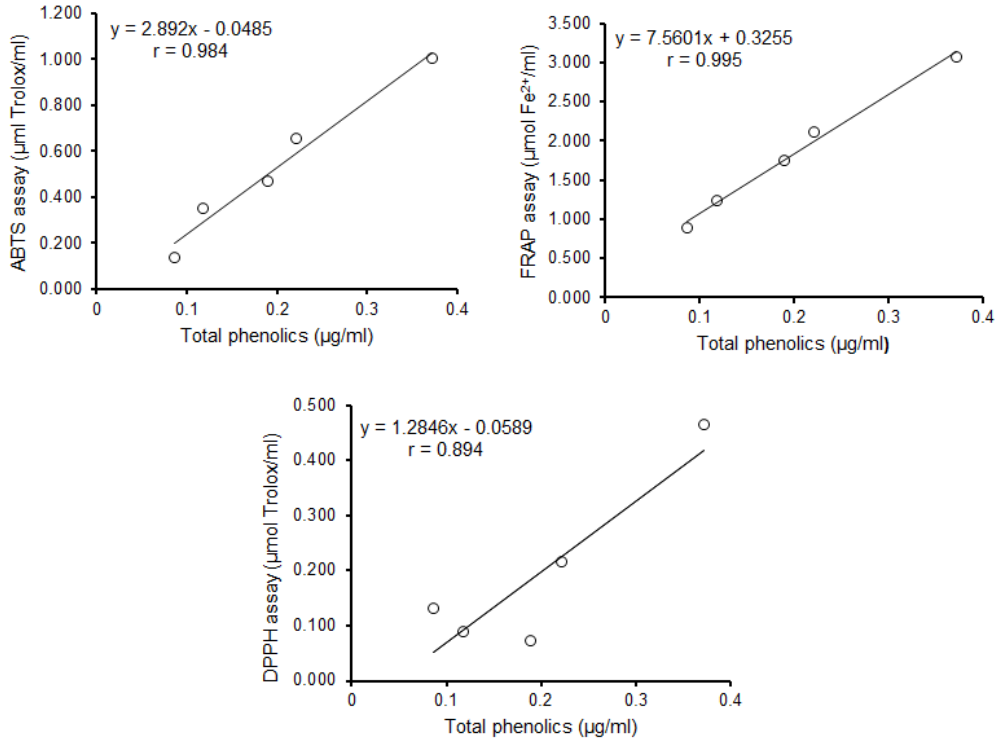

Fig. 3. Correlation between the content of phenolic compounds in kvass samples and their antioxidant activity.

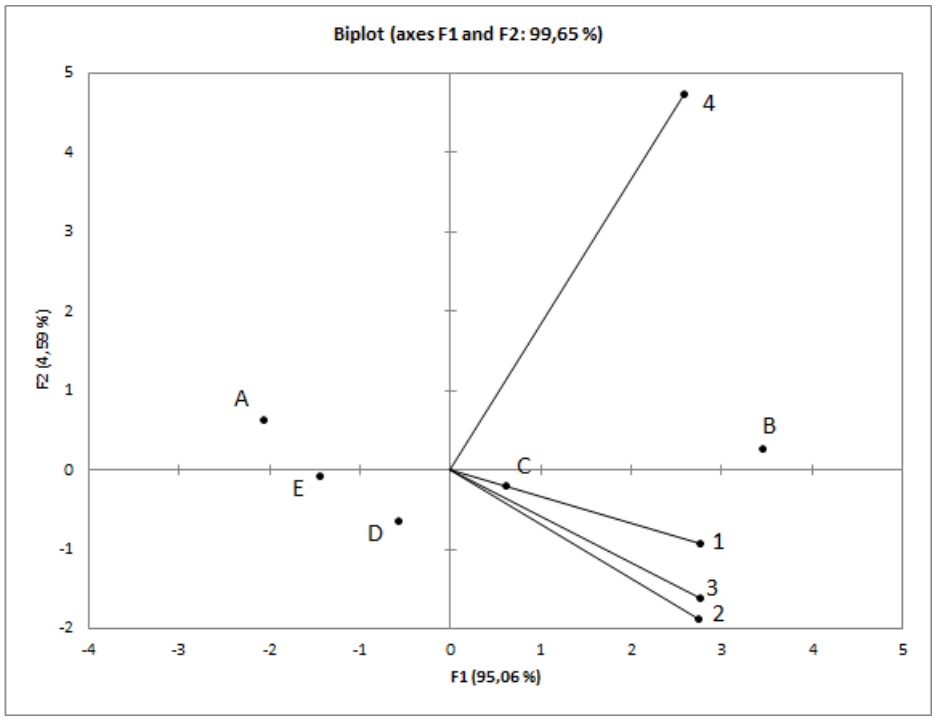

Fig. 4. Biplot of the principal component analysis (PCA). Distribution of samples: A – Obolon; B – Wileński; C – Gubernija; D – Eko-Natura; E – Brottrunk Biovegan and variables: 1 – Total phenolic content; 2 – ABTS; 3 – FRAP and 4 – DPPH.

In the principal component analysis (PCA) (Figure 4), the two first components accounted for 99.65% of the total variability between the kvass samples. The first principal component (F1) explained 95.06% of the total variance. Samples Obolon, Brottrunk Biovegan and Eko-Natura formed a homogenous cluster. It indicates similarities between the beverages. Two others: Gubernija and Wileński were separated due to the

highest antioxidant activity and total phenolics content.

CONCLUSIONS

Commercial kvass samples from Polish and German markets exhibit antioxidant potential confirmed by different chemical methods. The presence of several phenolic compounds has been confirmed using the HPLC method. The strong correlation was noted between the content of total phenolics and results of ABTS, FRAP, and DPPH assays.

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