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A COMPARATIVE STUDY ON ANTIOXIDANT PROPERTIES AND METAL CONTENTS OF SOME EDIBLE MUSHROOM SAMPLES FROM KASTAMONU, TURKEY

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Abstract:

In this work *Pleurotus ostreatus*, *Agaricus bisporus* and *Lactarius delicious* were used to determine and compare their antioxidant capacities and metal contents. The edible mushroom samples were collected from Kastamonu in the West Black Sea region of Turkey. The antioxidant capacity studies were performed by DPPH (1, 1diphenyl-2-picryl hydrazyl) radical scavenging method and were expressed as Trolox equivalents with spectroscopic measurements. TEAC (Trolox Equivalent Antioxidant Capacity) values were found 0.302, 0.557 and 0.251 µM/g for *Pleurotus ostreatus*, *Lactarius delicious* and *Agaricus bisporus*, respectively. All samples were analyzed by X-ray fluorescence (XRF) spectrometry to obtain the concentration of Cr, Mn, Fe, Ni, Cu, Zn, Ca, Pb, Na, Mg and K. While maximum and minumum metal contents of mushrooms were found as mg/g for Na (96-14.9), Mg (8.83-2.60), K (4.05-3.16), Ca (0.089-0.019) and Fe (0.128-0.099), the maximum and minumum contents of mushrooms were found for Cr (8-5), Mn (12-11), Ni (15-6), Cu (30-20), Zn (7-3) and Pb (3-1) as mg/kg. Metal contents were determined together with antioxidant capacity of all analysed mushrooms. It was observed that although the Fe, Ni, Ca, Na, and Mg contents of *Agaricus bisporus* were lower, it had got higher inhibition than the other mushroom species.

Keywords: Antioxidant capacity, Metal contents, *Agaricus bisporus*, *Pleurotus ostreatus*, *Lactarius delicious*, TEAC values, Kastamonu

Introduction

There are many reactive oxygen species and free radicals that are formed as a result of the oxidation process which is an important process in terms of energy production in biological systems. These reactive species take part in degenerative processes and functional changes associated with diseases like cancer, rheumatoid arthritis, cirrhosis etc (Babu & Rao, 2013).

Antioxidants are substances that may help the body to protect against various types of oxidative damage and can prevent oxidation by various mechanisms such as scavenging free radicals, chelating pro-oxidant metal ions, quenching secondary oxidation products, and inhibiting prooxidative enzymes (Gülçin, 2012; Rajalingam *et al.*, 2013; Bakır *et al.*, 2013).

Recently, naturally occurring antioxidants for use in foods or medicinal materials have started to replace some synthetic antioxidants which are being restricted due to their negative health effect. Mushrooms have natural antioxidans and they have long been consumed as a part of the normal diet in many European countries because of their unique taste and subtle flavor. At the same time mushrooms which are a rich source of nutrients on account of carbohydrate, protein, ascorbic acid, tocopherols, iron, zinc, selenium, sodium, chitin, fibres and minerals have many biologically active components that offer health benefits and protection against degenerative diseases (Baysal *et al.*, 2007; Ouzouni *et al.*, 2009; Yılmaz *et al.*, 2016). These functional properties of mushrooms are mainly due to their chemical composition. Many wild edible mushroom species are known to accumulate high levels of heavy metals. Therefore, many studies have been made on their metal content (Isildak *et al.*, 2004; Cocchi *et al.*, 2006). Intensive research has been carried out to detect and explain the presence and distribution of several heavy metals in edible mushrooms, in particular arsenic, cadmium, cesium, copper, iron, lead, manganese, mercury, selenium, rubidium, and zinc (Cocchi *et al.*, 2006).

The chemical compositions of mushrooms caused some changes in their antioxidant properties and also their metal contents are effective in the change of these properties. But investigations which display the changes of the antioxidant properties with their metal content are very few. The minerals can be accumulated in mushrooms, and this accumulation is generally species metabolism-dependent and also strongly affected by the chemical composition of the substrate from which mushrooms get their nutrients (Radulescu *et al.*, 2010).

Atomic absorption spectrometry (AAS) has been one of the most used techniques for elemental analysis; however, the suitability of X-ray fluorescence (XRF) technique has been established to be suitable to determine the elemental content in biological samples with the big advantage of being non–destructive (Carvalho *et al.*, 2005).

Agaricus bisporus and *Pleurotus ostreatus* preferred by the people because of easily and rapidly cultured, *Lactarius delicious* preferred for its flavor and these mushrooms are always have a comprehensive and regular trade in our country. Previously antioxidant activity wasn't been assessed with metal content together in these mushroom species which were collected from Kastamonu region. In this work we determined the antioxidant capacity in order to identify how changes their antioxidant capacity with concentration of heavy metals in these mushroom species. For this purpose, we calculated antioxidant capacity of mushrooms using DPPH (1, 1diphenyl-2-picryl hydrazyl) radical scavenging method to compare trolox equivalent capacity. We used XRF Spectrometry to find heavy metal concentrations in these edible mushrooms.

Materials and Methods

Study Field and Labratory Works

Pleurotus ostreatus colected from Bozkurt at May 2016 and *Lactarius delicious* collected at November 2016 from Devrekani in Kastamonu. *Agaricus bisporus* samples taken from Kastamonu University Mushroom Research and Application Center. Mushroom species was confirmed by Prof. Dr. Sabri Unal at Mushroom Research and Application Center, Kastamonu University.

All chemicals which were analytical grade provided from Sigma-Aldrich Co. LLC. In each stage deionized purity water was used. Absorbents was measured using a SHIMADZU the UVM-1240 UV-Visible spectrophotometer (Shimazu Corp., Kyoto, Japan manufactures) with a pair of identical quartz cuvette of 1 cm thickness at 517 nm. XRF measurements were made with X-Ray Fluorescence Spectrometer (Spectro Xepos II).

Determination of DPPH Activity

Free radical scavenging effects of trolox and mushroom extracts was performed by using DPPH method. DPPH radical available as commercially and one of the stable radicals that used in the antioxidant capacity and activity assay. Its ethanol solution was purple and gives the maximum absorbance at 515-517 nm. When reduced by antioxidants, its color turns lighter and progress of the reaction can be monitored with a spectrophotometer. The amount of antioxidant which required to reduce DPPH concentration by 50% is a commonly used parameter to measure the antioxidant activity and it is called IC_{50} (mg/mL) (Frankel & Meyer, 2000).

Preparation of mushroom extracts

Pieces of 1 gram were taken from each mushroom species and was pulverized in a porcelain crucible. Then they were dissolved in 10 ml, 75% ethanol solution. The mixture was filtered through a filtration cloth after waited 30 minutes at room temperature. The resulting homogenate centrifuged at 5000 rpm for 10 minutes (at 18ºC). The supernatant that received from this process centrifuged at 7500 rpm for 10 min (at 4ºC) again. The final supernatant was taken and used (100 mg / ml) for DPPH and XRF measurements (Pedraza-Chaverri, *et al.* 2004; Lee *et al.*, 2004).

Preparation of Trolox solution

100 mg Trolox ($C_{14}H_{18}O_4$) was taken and dissolved in 100 ml 75% ethanol ($4x10^{-3}$ M). Then $8x10^{-5}$ M concentration was obtained by diluting this solution.

Preparation of DPPH calibration solutions

123 mg DPPH ($C_{18}H_{12}N_5O_6$) was dissolved in 50 ml of absolute alcohol ($6.25x10^{-3}$ M). Then diluted from this solution and concentration of $1.25x10^{-3}$ M as well, $2.5x10^{-4}$ M and $5x10^{-5}$ M DPPH calibration solutions were prepared. Absorbance of the DPPH solutions were read and the calibration graph is obtained. And the calibration equation were shown for DPPH solutions at the concentration range $5\text{-}25x10^{-5}$ M.

For this study, first of different concentrated DPPH calibration solutions prepared with ethanol incubated for 15 minutes at room temperature and in the dark and then absorbance at 517 nm were recorded corresponding to the blank. In the same way ethanol -DPPH solution which prepared for control was used as a standard for sample studies.

Determination of Total Antioxidant Status

Preparation of Sample [TR + Ethanol + DPPH] system solution

The solution was prepared as follows: 3 ml (stock $2.5x\ 10^{-4}$ M) DPPH + X ml TR (Trolox) + (3-X) ml of absolute ethanol; total volume of 6 ml of the reaction mixture.

Preparation of Sample [Mushroom Extract + Ethanol + DPPH] system solution

The solution was prepared as follows: 3 ml (stock $2.5x\ 10^{-4}$ M) DPPH + X mL Mushroom Extract + (3-X) ml of absolute ethanol; total volume of 6 ml of the reaction mixture.

Percentage of radical scavenging activity is calculated by the following formula:

$$\% \text{ Inhibition} = [(C_0 - C_1) / C_0] \times 100$$

C_0: Concentration of control solution (no antioxidant added) and C_1: Concentrations of sample solutions (when antioxidant was present) (Huang *et al.*, 2005).

The IC_{50} value was determined from the graph slope "y = mx + c" formula that obtained from the graph for standard trolox and mushroom extracts (Mukherjee *et al.*, 2011).

Study of Metal Content

The collected mushroom samples dried in drying oven (NUVE KD 400) at 105ºC for 24 hours and then pulverized and stored in polyethylene bottles prior to analysis. These samples (1 g) were digested with 12 ml of HNO_3 (65%) and 4 ml of H_2O_2 (30%) in a microwave digestion system for 45 min and diluted to 20 ml with deionized water. Prepeared samples were analysed by X-Ray Fluorescence Spectrometer for three times, repeatedly (Mendil *et al.*, 2004).

Statistical Analysis

The relationship between Trolox and antioxidant concent of mushrooms were calculated using descriptive statistical analysis with Microcal Origin Pro 8.5.1 (Origin Lab. Corp., Northampton, MA, USA). Statistically significant effects were investigated using SPSS software (SPSS Inc., Chicago, IL, USA) for Windows version 13.

Results and Discussion

Antioxidant activity

In this study, we used DPPH radical quenching method and trolox which is a water-soluble antioxidant as a standart. Free radical scavenging effects of trolox and mushroom extracts was performed by using DPPH method. Therefore, we calculated calibration equation $y=7.62x10^3c-0.018$ ($R^2=0.999$) with the help of different DPPH concentrations. We use this calibration equation for calculating percent of inhibition of Trolox and mushroom species.

Decreasing absorbance values of samples that prepared with Trolox and mushrooms extracts gave remaining DPPH solution values so free radical scavenging activity. And we showed inhibition values and concentration equals of Trolox solution at various concentrations. To determine the (unitless) TEAC coefficient of each compound, the ratio of the slope **(*m*)** of the linear regression curve of the tested compound to that of Trolox was used:

$$TEAC = m_{compound} / m_{Trolox}$$

Then the calculated trolox equivalents can be used for comparative analysis of the antioxidant capacity of the various mushroom samples. As can be seen from Table 1, Concentration equality and TEAC (Trolox equivalent antioxidant capacity) values calculated by DPPH method for each mushroom species are illustrated. And we found TEAC values for, *Pleurotus ostreatus*, *Lactarius delicious* and *Agaricus bisporus* 0.302, 0.557 and 0.251 µmol /g, respectively.

Thus, we gave results for determination of antioxidant capacity of these fungal extracts as % inhibition in Figure 1. According to these results we obtained different antioxidant properties for the each fungal species. Although *Agaricus bisporus* generally showed the highest activity, we didn't observe a linear inhibition with increasing concentration. However, *Pleurotus ostreatus* and *Lactarius delicious* indicated a linear inhibition with increasing concentration.

And as a result, IC_{50} values that were calculated with DPPH method were found 4.51, 60.32, 21.35 and 8.33 mg/mL for trolox, *Pleurotus ostreatus*, *Lactarius delicious* and *Agaricus bisporus* respectively. Considering the IC_{50} values, in spite of the low concentration, It was observed that *Agaricus bisporus* has more potent antioxidant activity because of scavenging the same amount of free radical.

Onbaşılı *et al.* (2015) studied about antimicrobial, antioxidant activities and chemical composition of *Lactarius deliciosus* (L.) collected from Kastamonu province of Turkey. They found IC_{50}value ›17 for *Lactarius deliciosus* and we found that IC_{50} value of *Lactarius deliciosus* similarly.

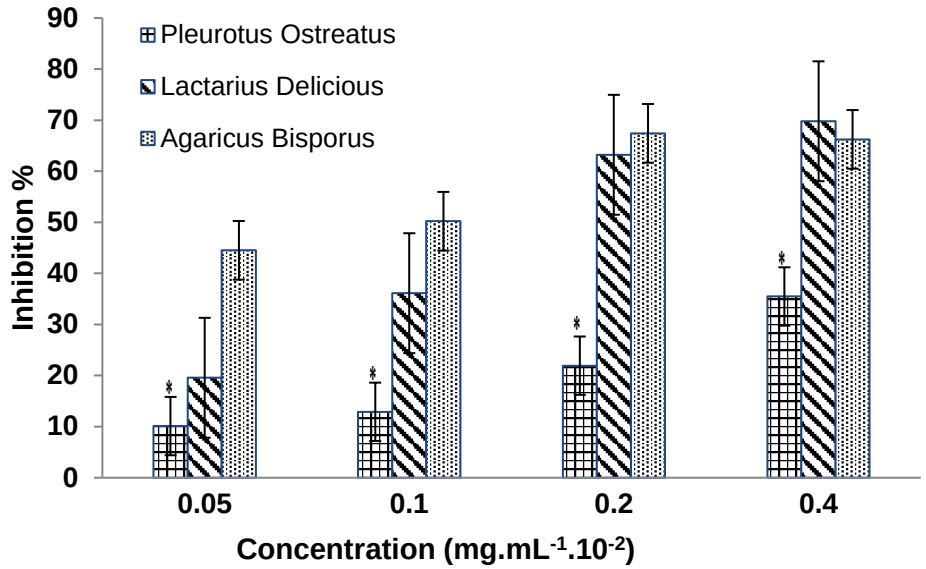

Figure 1. Antioxidant capacity of *Pleurotus ostreatus*, *Agaricus bisporus* and *Lactarius delicious* in different concentrations (measured by DPPH assay). The calculated results are given as mean ± SEM (standard error of the mean). *The statistical significance was accepted at $P<0.05$, (n=4).

Table 1. Calculated of TEAC (trolox equivalent antioxidant capacity) coefficients of *Agaricus bisporus, Pleurotus ostreatus* and *Lactarius delicious* by DPPH method

	Concentration (10^{-6}M)	Inhibition %	Concentration Equation	R^2	TEAC** c=(4.0–32.0) x10^{-6} M
Trolox	4	15.07	y=2.383x10^6c+7.097	0.995	-
	8	28.41			
	16	44.58			
	32	83.32			
	Concentration (mg.mL^{-1})*				
Pleurotus ostreatus	5	10.35	y=0.719c*+6.627	0.994	0.302
	10	13.08			
	20	21.90			
	40	35.12			
Agaricus bisporus	5	19.59	y=1.327c*+21.667	0.705	0.557
	10	35.75			
	20	62.21			
	40	68.61			
Lactarius delicious	5	43.94	y=0.598c*+45.016	0.523	0.251
	10	49.51			
	20	66.30			
	40	65.15			

Mushroom extract concentrations (c*): 5.0; 10.0;20.0;40.0 mg/mL (g/L)
TEAC of mushrooms (**): µmol of Trolox equiv per gram mushroom extract (µM of Trolox equiv./g)

Determination of metal contents

There have been many studies on the mechanism of antioxidative activities of phenolic compouns and flavonoids. At the same time there are many studies on the metal complexation with phenolic compounds such as flavanoids. Rice –Evans *et a.l* (1996) found a relationship between antioxidative activity and structure in flavanoids and they reported metal ions such as copper, iron, zinc, sodium and potasium effects. Nathan *et al.* (2009) showed that both Fe^{2+} and Cu^{+} perform Fenton-like reactions with H_2O_2, polyphenol compounds containing metal binding catechol and gallol groups have very different activities, depending on the metal ion. It is clear that iron-binding are important factors contributing to overall antioxidant activity for polyphenol compounds. And Khokhar *et al.* (2003) said that binding of iron to the flavonoid antioxidants can suppress the accessibility of the iron to oxygen molecules.

This study is a macro dimension work which is discussed in order to demonstrate statistically the effects of different antioxidant properties and the effect of accumulated metals in the fungal species to oxidation mechanism. Eleven metals (Na, Mg, K, Cu, Mn, Zn, Fe, Ca, Ni, Pb and Cr) and two metal oxides (Al_2O_3 and SiO_2) were determined in three mushroom species. Element concentrations and percent of the compounds of the mushroom species are presented in Table 2. According to the results, the most abundant elements were Na, Mg and K, respectively. These are followed by Fe and Ca. And the other minor ones were Cu, Ni, Mn and Pb.

The heavy metal concentration in the mushrooms are mainly affected by acidic and organic matter content of their ecosystem and soil .Toxic heavy metal (such as Pb, Cr, Cu etc.) concentrations of the investigated three mushrooms in this study were found at relatively low levels compared to those of the essential elements and therefore the results presented here were acceptable to human consumption at nutritional and toxic levels (Turkekul et al., 2004; Ayaz *et al.*, 2011).

In this study, the highest copper and manganese contents were found 30 mg/kg and 12mg/kg respectively. And These copper and manganese levels in mushrooms are in good agreement with other studies (Demirbaş, 2000; Işıloğlu *et al.*, 2001).

Table 2. Levels of elementel contents and metal oxides for three different mushroms species, dry weight (as µg /g) and (wt. %). Data are expressed as mean value ± standart deviation (SD).

Compound (wt.%)	*Pleurotus ostreatus*	*Agaricus bisporus*	*Lactarius delicious*
Al_2O_3	0.04 ±0.001	0.07 ±0.001	0.12 ±0.001
SiO_2	0.03 ±0.001	0.01 ±0.001	0.01 ±0.001
Element (mg/kg)			
Na	96000 ±9500	87000 ±8600	14980 ±480
Mg	8830 ±210	8500 ±200	2600 ±140
K	3162 ±29	4057 ±30	3623 ±26
Cr	6 ±1.0	8 ±1.0	5 ±1.0
Mn	11 ±1.0	12 ±1.0	12 ±1.0
Fe	128 ±4.0	121 ±4.0	99 ±3.0
Ni	15 ±1.0	12 ±1.0	6 ±1.0
Cu	30 ±3.0	25 ±2.0	20 ±2.0
Zn	5 ±1.0	7 ±1.0	3 ±1.0
Ca	89 ±7.0	83 ±6.0	19 ±3.0
Pb	1 ±0.5	3 ±1.0	2 ±1.0

In the previous works Mendi *et al.* (2004) studied about determination of trace elements on some wild edible mushroom samples from Kastamonu, Turkey. They gave concentration results of nine trace elements in eight mushroom species and found iron, manganese and zinc contents of mushrooms were higher than ours results. But they found that the similar metal contents order for *Agaricus bisporus* and *Lactarius delicious* in terms of metal contents.

Statistically correlation coefficients were found ($r>0.977$) at ($p<0.05$) significant level between metal concentrations for all mushrooms. Positive correlations were obtained between sodium and chromium (r = 0.68), sodium and nickel (r = 0.97), sodium and copper (r = 0.91), magnesium and chromium (r = 0.72), magnesium and iron (r = 0.98), magnesium and copper (r = 0.88), iron and nickel (r = 0.99), iron and copper (r = 0.95) and copper and zinc (r = 0.50) and negative correlations were found between potasium and copper (r=-0,51), manganese and iron (r = -0.68), manganese and nickel (r = -0.75) and manganese and copper (r = -0.86).

On the other hand, we showed the distribution of some element species in mushrooms species in Figure 2. Although Fe and Ca concentrations were higher in *Lactarius delicious, Pleurotus ostreatus*, they were lower in *Agaricus bisporus*. Similarly Cu concentration followed by the same sort of, but according to mushroom spices the difference was quite small. In this study though the most abundant elements were Na and Mg for each mushroom spices, they were less in *Agaricus bisporus* than the others. In the case of heavy metal oxide, the most abundant was Al_2O_3 in *Agaricus bisporus.*

In an other work Özyürek et al. studied about antioxidant/antiradical properties of microwave-assisted extracts of three wild edible mushrooms (*Terfezia boudieri* Chatin, *Boletus edulis* Bull., *Lactifluus volemus* (Fr.) Kuntze) in different regions of Anatolia-Turkey (Özyürek *et al.*, 2014).

Ayaz *et al.* (2011) studied about the nutritional content of eight edible mushrooms (*Boletopsis leucomelaena* (Pers.) Fayod, *Hydnum repandum* L., *Laetiporus sulphureus* (Bull.) Murrill, *B. edulis, Armillaria mellea* (Vahl) P. Kumm., *Macrolepiota procera* (Scop.) Singer, *Lactarius piperatus* (L.) Roussel and *L. quietus* (Fr.) Fr.) collected from East Black Sea region in Turkey.

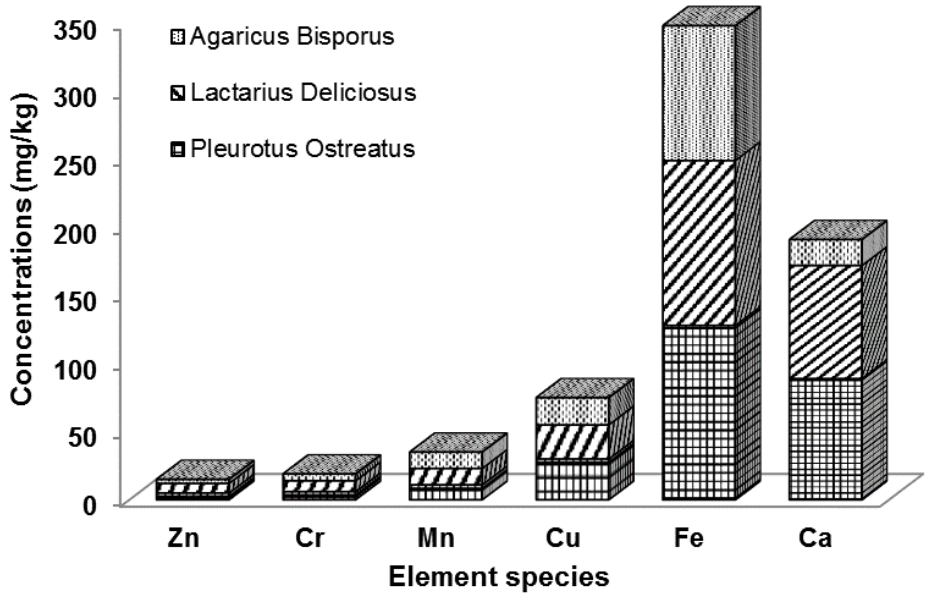

Figure 2. Distribution of element species (Ca, Fe, Cu, Mn, Cr and Zn) in *Agaricus bisporus*, *Lactarius delicious* and *Pleurotus ostreatus*.

Based on the results obtained, the methanolic extract of *Pleurotus eryngii* (DC.) Quél. collected from city center revealed the highest DPPH radical scavenging activity and reducing power, while the highest total phenolics and total antioxidant status was determined in *P. eryngii* collected from Pulumur (Çıkçıkoğlu *et al.*, 2012).

Kumamoto *et al.* (2001) and Rice –Evans *et al.* (1996) found that some ions were able to inhibit antioxidant activity like iron. Kumamoto *et al.* (2001) examined antioxidative activity of (-)-epigallocatechin gallate (EGCG) in the presence of thirteen kinds of metal ions. The antioxidative activity of EGCG was increased by Cu^{+2} and Mn^{+2}, and on the contrary inhibited by Fe^{+2}. And our results were compliance with these studies.

Conclusions

Considering together IC_{50} and metal contents, we saw that *Agaricus bisporus* has got the highest antioxidant activity and lower Fe and Ca concentrations than the others. According to our study a relationship was found between the increase of Fe, Ca, Na, and Mg concentrations and antioxidant activity in the opposite direction. But we didn't compare relation between phenolic antioxidants and metal contents in the mushroom species. Therefore we are going to study polyphenolic contents of the mushroom species to make a definite judgment in an other work.

Abbreviations

AAS	Atomic absorption spectrometry
C_0	Concentration of control solutions
C_1	Concentration of sample solutions
DPPH	1, 1diphenyl-2-picryl hydrazyl
EGCG	(-)-epigallocatechin gallate
IC_{50}	The half maximal inhibitory concentration
SEM	Standard error of the mean
SD	Standart deviation
TEAC	Trolox Equivalent Antioxidant Capacity
XRF	X-ray fluorescence spectrometry

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