Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XVI, Issue 1- 2017, pag.5 - 12

FOOD AND ENVIRONMENT SAFETY

STUDY OF THE EVOLUTION OF MICRO- AND MACROELEMENTS DURING THE WINEMAKING STAGES: THE IMPORTANCE OF COPPER AND IRON QUANTIFICATION

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Received February 15th 2017, accepted March 27th 2017

Abstract: *Knowledge of the concentration of mineral elements from winemaking products, particularly from the final product is important because of their influence on wine quality. Some metal ions such as iron and copper can induce haze formation and changes in the sensory proprieties of wine. The presence of heavy metals in wine is due to different factors including vineyard soil, agricultural practices (the use of fertilizers and pesticides), and can be at the same time a result of environmental pollution. In addition, the acidity of wine and grape must (freshly pressed grape juice) can dissolve Cr, Cu, Ni, and Zn from winemaking equipment like pumps and taps. As wine is the most widely consumed alcoholic beverage, analytical control of mineral elements content is required during the whole process of wine production, from the grapes used to the final product. In this study the content of micro- and macroelements in grape pomace, yeast sediment, grape must and wine was determined by inductively coupled plasma-mass spectrometry (ICP-MS). Samples of winemaking products originating from five grape varieties were analyzed in four forms in order to determine to what measure the values varied the PCA (Principal component analysis). The obtained results using PCA highlighted major differences in the content in trace elements between samples.*

Keywords: grape pomace, grape must, ICP-MS, wine, yeast

1. Introduction

The content in mineral elements of food products has been extensively studied, in recent papers [1-4]. The analysis of mineral elements in alcoholic beverages [5] is particularly centered on the heavy metal content. Among alcoholic beverages, wine has been the most popular and widely-consumed worldwide since early civilization [6]. According to the International Organization of Vine and Wine, the worldwide production of wine was estimated at 259 Mhl in 2016.

Wine is a product obtained exclusively through the alcoholic fermentation of fresh grapes. The final beverage is a complex matrix containing water, alcohol, sugar, and a great variety of components, organic as well as inorganic ones [7].

Wine quality depends on the chemical composition, which is influenced by both geographic factors (climate, soil, grape variety and culture) and factors related to the production process (winemaking, transport and storage). Inorganic ions concentration in wine is of great interest [8], as in some cases it can have a major impact on the quality of the final product. Mineral elements are absorbed from the soil through the vine roots and are accumulated in the cellular walls, skin and seeds of the grape. During winemaking, the minerals from grapes pass into wine. Therefore, the mineral composition of wine reflects its origin and development, making it unique and identifiable. Oroian (2015) achieved an authentication of Romanian white wines varieties in terms of the content in 27 mineral elements [9]. It contributes significantly to wine's sensory characteristics, affecting color, clearness, flavor and aroma [10].

Most of the minerals are important to the alcoholic fermentation: calcium, potassium, magnesium, and sodium maintain an adequate pH and ionic balance stabilizing the cellular metabolism of yeasts [11]. Minerals in lower concentration such as copper, iron, manganese, vanadium, zinc are also favorable for yeasts, particularly *Saccharomyces cerevisiae*. Copper alongside iron and manganese can cause sensory quality changes after bottling and influence the stability in old wine. The presence of high levels of both Fe and Cu leads to the formation of hazes in wine [12]. This undesirable oxidation process has an impact on the commercial acceptance of wine and can induce potentially toxic effects [13]. In this context, measurement of metal content is essential to provide the final product with high quality.

The mineral constituents of wine can be assessed by several analytical methods. Atomic spectrometry techniques such as inductively coupled plasma-mass spectrometry (ICP-MS) [14], ICP atomic emission spectrometry (ICP-OES) [15], and flame atomic absorption spectrometry (FAAS) have been extensively used for the elemental analysis of wine [16, 17].

The aim of this paper was to determine the metal ions in the winemaking process, namely grape pomace, yeast, grape must, and wine, and to study the changes in the concentration of these elements during winemaking.

2. Materials and methods

Samples. Samples of winemaking products (grape pomace, yeast, grape must, and wine) obtained from five different grape varieties were analyzed by ICP-MS. The grape varieties were Feteasca Neagra, Merlot, Chasselas, Riesling, Ottonel. All grapes used in winemaking originated from the region of Focsani, Romania.

Sample preparation. 5 grams of grape pomace and yeast were mineralized in an electric furnace at a temperature of 600°C, for 6 hours. The resulted ash was transferred into a 50 mL volumetric flask, where it was dissolved by adding a mixture of nitric acid and deionized water until the concentration of nitric acid in the solution was of 1% [18]. The elemental analysis of grape must was conducted according to Toaldo *et al.* (2013). An aliquot of 500 µL of sample was diluted to 10 mL with 0.14 mol/L nitric acid, then directly analyzed by ICP-MS [19].Wine samples were prepared according to Oroian (2015).

Reagents. The reagent used was of high purity grade: double deionized water (18 MΩ cm resistivity) produced by a water purification system (Thermofisher, Germany) was used in all solutions. Samples were digested with concentrated nitric acid (65% HNO_3, Sigma Aldrich, Germany) and hydrogen peroxide (30% H_2O_2 pure, Sigma Aldrich, Germany).

Apparatus. The determination of 12 elements was performed in a mass spectrometer with inductively coupled plasma, (ICP-MS) Agilent Technologies 7500 Series (Agilent, USA).

Statistical Analysis. Statistical analysis, PCA and ANOVA, was performed using XLSTAT 2016.

3. Results and discussion

In this study the content of grape pomace, yeast sediment, grape must and wine in the following mineral elements: V, Mn, Fe, Ni, Au, Hg, Mg, Al, Ca, Cu, Zn, and Cr were determined.

To estimate the concentration of these elements in the sample following equation was used:

$$C = \frac{C_m x V_t}{V_m}$$

Where:

C = Concentration of the element in the sample;

C_m = Concentration of the elements in the diluted sample;

V_t = Final volume of the measurement solution, in ml;

V_m = Aliquot volume of wine, in mL [20].

The determined concentrations of micro- and macroelements are presented in Table 1. As it can be observed, magnesium was the most abundant mineral element, with concentrations that varied from 43.25 to 298.69 mg/L. High levels of calcium were also determined in all the samples analyzed. For the Merlot grape variety, grape pomace, must, yeast and the final sample of wine had a significantly higher content of magnesium and calcium.

The grape variety Merlot, alongside Feteasca Neagra had a remarkable content of copper and zinc. For Feteasca Neagra variety, the copper content ranged between 1.7 mg/L in wine and 41.1 mg/L in pomace. Compared to this, the copper content of grape pomace from Merlot variety was slightly higher (44.3 mg/L). Zinc concentration was comparable in these two grape varieties, especially in pomace and wine. Aluminum and chromium are found in an increased concentration in all the samples analyzed.

Among microelements, nickel and vanadium displayed significant variation of concentration during different stages of winemaking. The main microelement in winemaking products, nickel was determined in higher concentration in the Merlot variety. In contrast, the other red grape variety analyzed in this paper had a notable content of iron. Finally, the winemaking products obtained by processing varieties of white grapes (Chasselas, Riesling and Ottonel) had greater levels of Au.

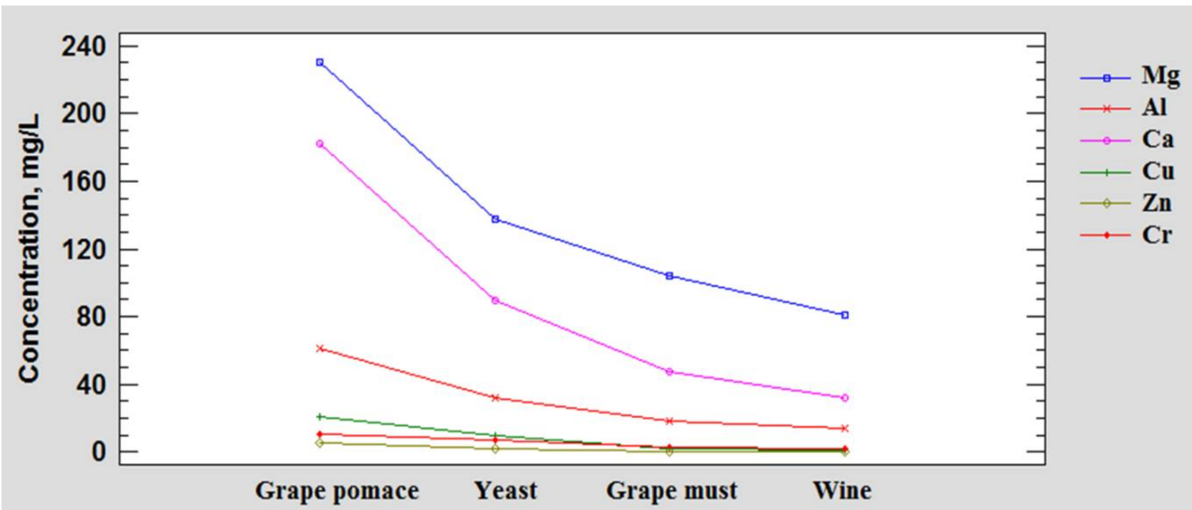

Fig. 1. Major mineral elements in winemaking products

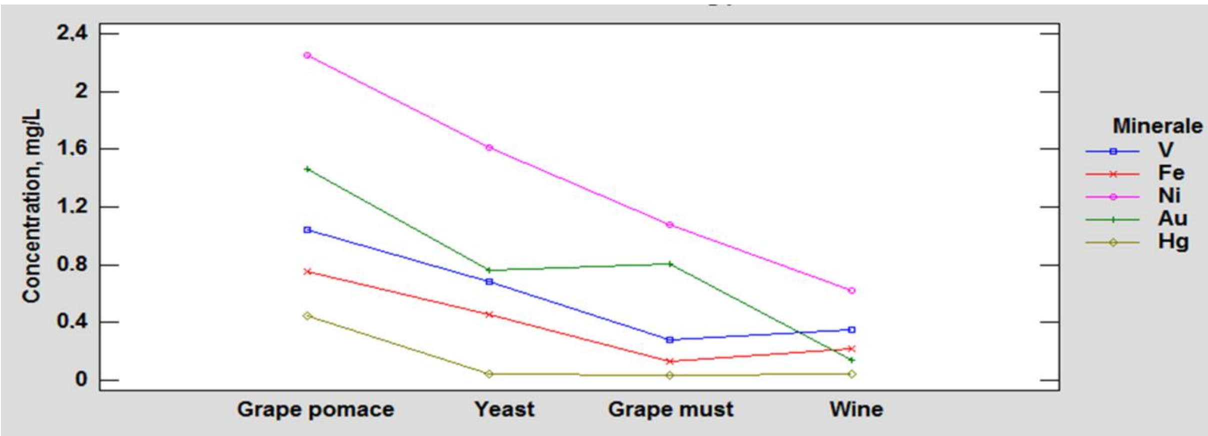

Fig. 2. Minor mineral elements in winemaking products

Multifactorial ANOVA was performed to evaluate the influence of interactions between factors (mineral element, analyzed sample and grape variety) on the concentration in minerals. Five grape varieties were analyzed in the mentioned forms, and the PCA (principal component analysis) was used to determine to what measure the values varied.
Of the major mineral elements in winemaking products, magnesium and calcium had the highest concentrations in all the samples analyzed, irrespective of the grape variety (Fig. 1). During the mechanical processes of crushing and pressing a proportion of minerals contained by grapes passes into must, while a large quantity of these elements remains in the pomace. With the removal of yeast sediment, the mineral elements concentration decreases and therefore the final product (wine) has the lowest mineral content.
A similar variation in the concentration determined in winemaking products was also observed for minor mineral elements (Fig. 2). Compared to other minerals, Au and Hg were found in lower concentration in yeast sediment samples.
Multifactorial ANOVA showed a significant interaction between the mineral element and grape variety ($p < 0.001$).

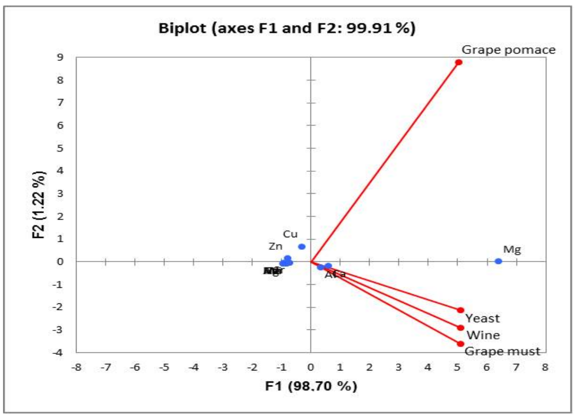

Fig. 3. PCA for Fetească Neagră

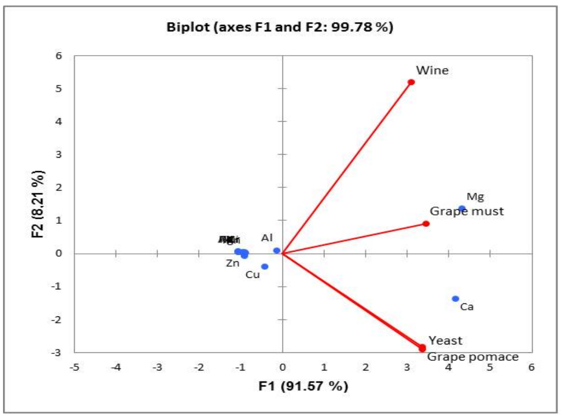

Fig. 4. PCA for Merlot

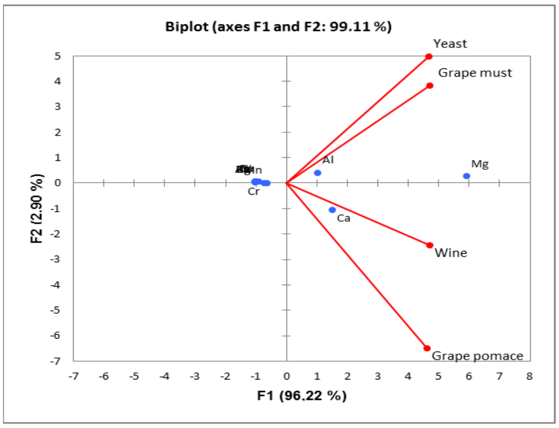

Fig. 5. PCA for Chasselas

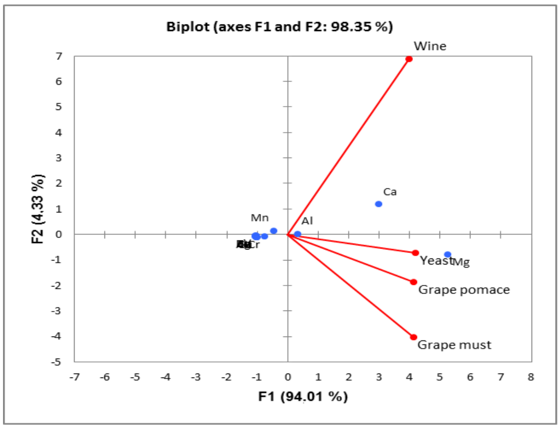

Fig. 6. PCA for Riesling

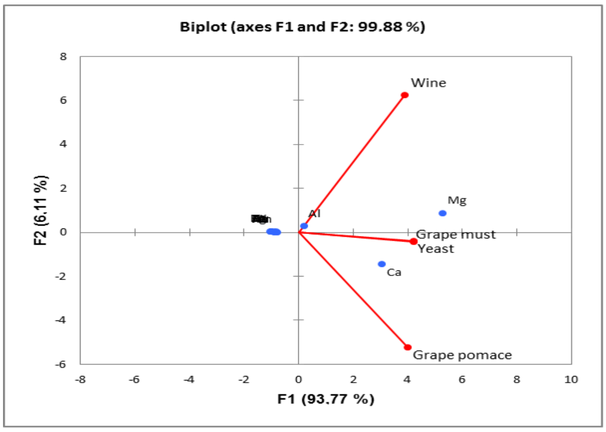

Fig. 7. PCA for Ottonel

Principal component analysis (Fig. 3-7) indicated positive correlations between the main mineral elements - Ca, Mg and Al and the four samples analyzed. The other minerals determined are displayed in opposed quadrant, and are found in lower concentrations.

Of particular interest were the concentrations of copper and iron. It is known that the presence of copper and iron ions can induce haze formation, which consequently leads to changes in the organoleptic properties of wine. The iron and copper content of the samples is presented in Fig. 8-9.

Concentrations of around 0.5 mg/L copper and 10 mg/L iron or higher can cause a metallic taste, haze, browning and other undesirable effects on wine. Its presence in higher concentrations in the must can even have an impact on the alcoholic fermentation, as it has been reported on several occasions, being capable of inhibiting the growth and development of several groups of certain naturally-occurring microorganisms.

According to the data presented in Table 1, the copper content of wine varied from 0.35 mg/L (Riesling) to 1.70 mg/L (Feteasca Neagra). The maximum level of copper specified in the European wine regulations and the compositional guidelines set by the International Organization of Vine and Wine is of 1 mg/L. On the other side, it is generally accepted that levels above 0.5 mg Cu/L are very likely to induce copper casse. Based upon this consideration, all the samples of wine analyzed excepting that of Riesling variety are susceptible to this type of oxidation.

Table1.

Concentrations of micro- and macroelements in winemaking products [mg/L]

Grape varieties	Analyzed samples	Hg	Au	Ni	Fe	Mn	V	Cr	Zn	Cu	Ca	Al	Mg
Feteasca Neagra	Grape pomace	0.05	0.18	1.73	0.97	2.04	0.56	5.53	10.92	41.10	27.20	20.59	161.09
	Yeast	0.04	0.19	1.04	0.70	1.97	0.54	4.83	0.39	6.69	27.19	20.50	106.71
	Grape must	0.01	0.19	1.30	0.07	1.86	0.12	1.89	0.24	1.74	20.84	20.82	101.19
	wine	0.01	0.04	1.07	0.50	1.12	0.50	1.68	0.32	1.70	20.93	17.26	105.57
Merlot	Grape pomace	0.02	0.07	4.55	0.48	8.44	0.80	6.37	11.34	44.34	212.03	35.41	142.33
	Yeast	0.11	0.36	4.04	0.33	4.79	0.55	3.22	7.74	31.37	211.73	30.21	140.05
	Grape must	0.07	0.10	1.48	0.28	3.92	0.55	5.14	0.75	2.82	98.54	12.78	114.36
	wine	0.04	0.14	1.48	0.05	1.47	0.09	1.60	0.21	1.35	38.11	16.15	102.86
Chasselas	Grape pomace	0.03	1.07	1.13	0.98	26.25	1.86	20.84	1.80	6.96	197.46	101.71	296.48
	Yeast	0.02	0.36	1.16	0.36	10.58	0.78	6.87	0.23	5.30	37.11	72.41	170.14
	Grape must	0.02	0.30	1.32	0.17	5.23	0.43	3.60	0.08	1.24	23.01	31.68	105.81
	wine	0.03	0.44	0.20	0.13	3.83	0.38	3.52	0.09	0.68	36.98	12.51	92.81
Riesling	Grape pomace	2.10	2.92	3.00	0.79	20.00	1.45	14.15	1.32	5.88	131.97	109.08	252.30
	Yeast	0.02	0.08	0.81	0.64	12.76	1.09	16.73	0.62	2.22	107.47	17.78	178.35
	Grape must	0.02	1.89	1.07	0.08	11.69	0.13	2.21	0.27	1.50	60.98	14.33	151.86
	wine	0.01	0.04	0.07	0.37	9.07	0.75	1.67	0.14	0.35	52.63	12.70	43.25
Ottonel	Grape pomace	0.01	3.07	0.87	0.54	7.73	0.55	7.10	1.84	7.19	343.29	37.14	298.69
	Yeast	0.02	2.80	1.00	0.25	6.07	0.43	4.44	0.34	1.78	63.11	19.39	92.78
	Grape must	0.07	1.56	0.21	0.06	2.37	0.14	1.79	0.28	1.22	33.34	11.75	48.31
	wine	0.00	0.04	0.28	0.02	0.54	0.03	0.70	0.28	1.13	13.00	11.05	59.68

Unlike the concentration of copper. iron did not exceed the maximum level allowed and therefore a haze formation caused by this metal is not posible. In the case of copper the increased content could be attributed to prolonged use copper-containig fungicide and particularly Bordeaux mixture (copper sulphate).This scenario is highly likely due to the fact that all grapes used in this study originated from a vineyard which is known to require this type of treatment in order to reach the desired quality and production. Moreover, the traditional winemaking process used in this case din not imply a removal of copper and iron through the addition of stabilizing agents (e.g. potassium ferricyanide).

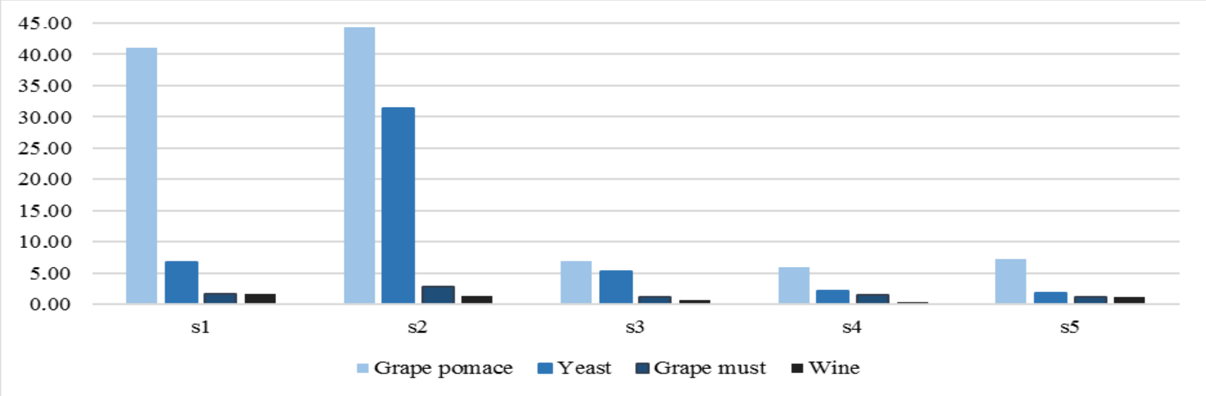

Fig. 8. Copper content in analyzed samples [mg/L], S1-S5Grape varieties (S1-Feteasca Neagra, S2- Merlot, S3-Chasselas, S4-Riesling, S5-Ottonel)

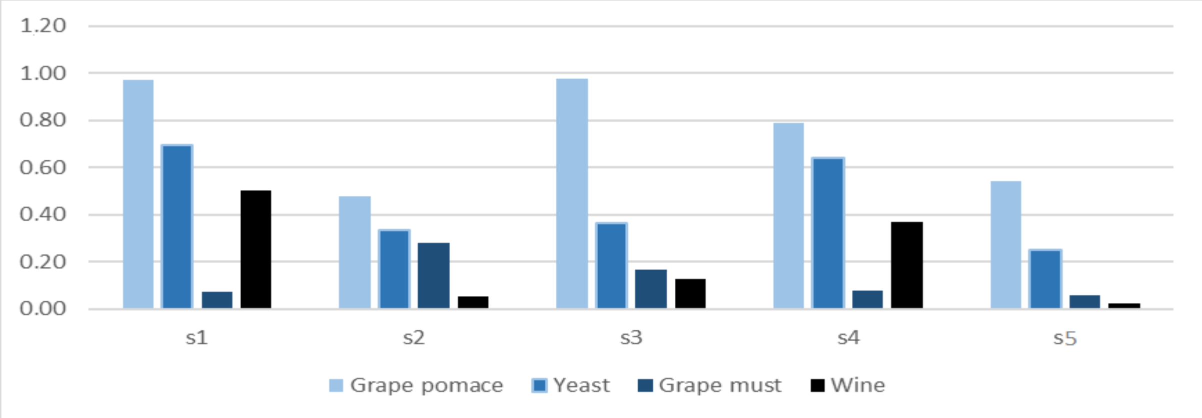

Fig. 9. Iron content in analyzed samples [mg/L],S1-S5 Grape varieties (S1-Feteasca Neagra, S2- Merlot, S3-Chasselas, S4-Riesling, S5-Ottonel)

4. Conclusions

The results of this study show a decrease in the concentration of mineral elements in the winemaking process from grape pomace, must, yeast sediment to wine. Multifactorial ANOVA indicated a significant interaction of the mineral element with the grape variety ($p < 0.001$).

PCA analysis highlighted a strong correlation between the elements with higher concentration and the corresponding winemaking products.

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