

Chemical and Sensory Properties of Chardonnay Wines Produced in Different Oak Barrels

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Abstract—French oak and American oak barrels are most famous all over the world, but barrels of different origin can also be used for obtaining high quality wines. The aim of this research was to compare the influence of different Slovenian (Croatian) and French oak barrels on the quality of Chardonnay wine. Grapes were grown in the Croatian wine growing region of Kutjevo in 2015. Chardonnay wines were tested for basic oenological parameters (alcohol, extract, reducing sugar, SO₂, acidity), total polyphenols content (Folin-Ciocalteu method), antioxidant activity (ABTS and DPPH method) and colour density. Sensory evaluation was performed by students of viticulture/oenology. Samples produced by classical fermentation and ageing in French oak barrels had better results for polyphenols and sensory evaluation (especially low toasting level) than samples in Slovenian barrels. All tested samples were scored as a “quality” or “premium quality” wines. *Sur lie* method of fermentation and ageing in Slovenian oak barrel had very good extraction of polyphenols and high antioxidant activity with the usage of authentic yeasts, while commercial yeast strain resulted in worse chemical and sensory parameters.

Keywords—Chardonnay, French oak, Slovenian oak, *sur lie*.

I. INTRODUCTION

CHARDONNAY is the most distributed grapevine variety all over the world unlike other white varieties of *Vitis vinifera* L. [1]. It is an extremely flexible variety that has adapted to different regions with varied weather and soil characteristics. Somewhat uniquely among white wines, Chardonnay lends itself to a wide variety of production styles, which can be tailored to the target market [2].

The production of quality wine involves a maturation step in wood barrels. Barrels have been widely used to age wines for centuries and most of them are made of oak wood. There are lots of places to grow barrel-worthy oak trees such as the former Soviet-style republics of central and Eastern Europe with Hungary in the first place, but also Russia, Romania, Croatia etc. All these areas have been making oak wine barrels for hundreds of years. The oak species most commonly used in barrel making are *Quercus alba*, also known as American oak, and *Quercus petraea* and *Quercus robur* which grow in Europe, the most popular being French oak. Croatian oak has traditionally exported in other European countries for years, but its influence on wine has not been widely explored.

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According to the FAO (Food and Agriculture Organization of the United Nation) Slovenian (eastern Croatia) oak is a high quality wood widely used in the traditional and classic Italian wines of the Veneto, Tuscany and Piedmont. Slovenian oak gives a less intense flavour than French oak, sweeter aromas and a less structured wine. Using the Slovenian oak rather than French oak makes micro-oxygenation (responsible for polymerization of tannins and avoid reductive flavours) less intensive and wine needs more time for obtaining the final quality during ageing [3]-[5]. Chira and Teissedre [6] investigated chemical and sensory properties of wines matured in three different oak barrels (Slovenian, American and French oak barrels). They reported that the forest origin of wood induced important changes in chemical composition of the wine, especially whiskey lactone and eugenol concentration. Ellagitannin concentration in Slovenian barrels was found to be halfway between French *Q. robur*, French *Q. petraea* and the American *Q. alba*. Spicy and overall woody notes grew from American oak to Slovenian oak and finally to French oak. Additionally, sweetness decreased from American oak to Slovenian and finally to European. Barrels made from Slovenian oak wood have an intermediary place between American oak and French oak. Moreover, Slovenian oak wood from *Q. robur* and *Q. petraea* is considered suitable for barrel production for high quality wines [6].

During wine aging, several processes considerably improve sensorial complexity. Aging in wood changes the colour, the polyphenolic profile and the aroma of wine but such wines are less rich in floral and fruity notes [7]. There are two main motives for wine aging in oak wood: the transfer of oak aroma volatile compounds and astringency-related phenolic compounds to wine, as well as controlled oxidation of certain compounds by atmospheric oxygen, resulting in a reduction of astringency and changes in colour. Still, wine should not be kept in barrels any longer than the moment it has reached its maximum quality [8], [9].

Many changes in chemical composition of wine aged in oak barrels depend on the level of wood toasting. Toasting represents a very important process, which gives specific characteristics to wines [10], [6]. During heat treatment, modification of wood chemical composition is induced by degree of toasting. The degradation of different components will contribute to the raise of volatile compounds level in wine [6]. Nevertheless, the use of different fermentation and aging conditions (i.e. ageing on lees, different origin of oak barrels, different toasting level, respectively), have not been systematically studied and compared, although they are often practically experimented by wine operators. The ageing on

lees or *sur lie*, as an oenological practice for quality wine production, is performed by letting wine in contact with resting yeast cells, is of growing interest with respect to the traditional one (ageing in stainless steel tanks without lees). The ageing on lees is generally coupled with use of barrels, since the wood allows oxygen exchange, which limits reducing defects promoted by the presence of lees [11]. Also, this winemaking practice facilitates an increase in the mouthfeel and the body of white and red wines, as well as their aromatic persistence [12]. There are several benefits in winemaking achieved by ageing on lees such as reducing the proteins in white wines, protecting wine from tartaric acid precipitation and favouring the growth of lactic bacteria. However, ageing on fine lees presents some risks for wine quality related to the appearance of sulphur, animal doors and metal tastes, the possible production of acetic acid and biogenic amines due to the metabolism of citric acid and amino acids [13]. On the other hand, during fermentation in stainless steel tanks there is no interaction between the tank material and the wine because stainless steel is a stable and inert material. Gonzalez-Marco et al. [14] studied the influence of an alcoholic fermentation container on the formation of volatile compounds in quality Chardonnay wines. The results of their work revealed that the type of container influences the fermentation bouquet to an important extent. This could be explained by the fact that the wood from new oak barrels, unlike stainless steel tanks, is a porous material, which interacts during fermentation. Herjavec et al. [3] described that fermentation in Croatian oak barrels positively influenced the quality of Chardonnay and Sauvignon wines. In comparison with stainless steel tank-fermented wines, these wines were characterized by a more complex flavour and aroma intensity.

The history of wine quality evaluation is longer than for any other food product. With an increasing consumer demand for better wines, competition of wine producers increases as well as development of appropriate statistical procedures for the analysis of sensory data. Lastly, sensory evaluation of wines gives final judgement of wine quality [15].

It is necessary to understand the impact of different factors to produce best quality wines. Therefore, the aim of this research was the utilization of different fermentation (*sur lie* and classical fermentation) and ageing conditions (ageing in Slovenian and French oak barrels on lees and without lees) in Chardonnay wine production. To the best of our knowledge polyphenols and antioxidant activity of wines, which are in the focus of this research, aged in Slovenian oak has not been researched yet. Wines were also tested for other chemical parameters, colour and sensory quality.

II. MATERIAL AND METHODS

This research was conducted in the vineyards and cellar of Polytechnic in Požega (Croatia). Grapes of Chardonnay (*Vitis vinifera L.*) variety were used for the experiment. Vineyard is situated on the southern slopes of Papuk mountain at an altitude 250 m. It belongs to the Eastern Slavonia region, Kutjevo sub-region.

Grapes were manually harvested on September 2, 2015. The amount of grapes after the harvest was 4775 kg. The sugar level in the grapes was 97 °Oe. After crushing and pressing of the grapes (mechanical press), must is put in tanks for precipitation. After 48 hours, clear must (3450 L) is decanted into fermentation barrels and the starter cultures of yeasts were added (25 g/100 L). Besides commercial starter cultures (SIHA Element), fermentation is also done by authentic yeasts. Fermentation was done in two different ways: classical fermentation in stainless steel tanks and by *sur lie* method. After the end of fermentation wine is transferred to different barrique barrels (Table I) for ageing. After six months in barrique barrels wine is bottled and analysed.

By combination of treatments following samples were obtained:

TABLE I
LIST OF SAMPLES

Sample	Technology	Barrel
W1	Sur lie, commercial yeast	Barrique-new, Slovenian oak, MT
W2	Sur lie, authentic yeast	Barrique-new, Slovenian oak, MT
W3	Stainless steel fermentation, commercial yeast, wood ageing	Barrique-new, French oak, MT
W4	Stainless steel fermentation, commercial yeast, wood ageing	Barrique-new, French oak, LT
W5	Stainless steel fermentation, commercial yeast, wood ageing	Barrique-new, Slovenian oak, MT
W6	Stainless steel fermentation, commercial yeast, wood ageing	Barrique-2 years used, Slovenian oak, HAT

A. Basic Oenological Parameters Determination

Alcohol strength by volume, extract, total acidity, reducing sugar, pH and ash were determined according to the official OIV methods [16]. SO₂ was determined according to the Ripper procedure [17]. Determination of each parameter was done in two repetitions.

B. Total Polyphenols Determination

Polyphenols were determined according the Folin-Ciocalteu method with modifications [18]. An aliquot of wine (200 µL) was mixed with 2 ml water and 100 µL Folin-Ciocalteu reagent (Kemika, Croatia). The mixture was allowed to equilibrate for five min, and then 300 µL of sodium carbonate solution (20%) was added. After incubation at room temperature in dark for 30 min, the absorbance of the mixture was read at 725 nm (Camspec M501, UK). Acidified methanol was used as a blank. Total polyphenols were determined with three replications. Gallic acid (Carlo Erba reagents, Italy) was used as a standard (calibration curve $y=0.1602x - 0.0008$, $R^2=0.9998$), and results were expressed in mg of Gallic acid equivalents per litre).

C. Antioxidant Activity Determination (ABTS)

ABTS^{•+} radical was obtained by mixing 7.4 mM ABTS (Fluka, Switzerland) solution and 2.6 mM solution of ammonium persulfate in 1:1 ratio. The solution was left in the dark through the night in order to develop stable radical, and then the radical solution was diluted with ethanol in 2:70 ratio to obtain absorbance approximately 1.100 (A_{ABTS}). An aliquot

of wine, was mixed with 3.2 ml of diluted ABTS^{•+} radical. After incubation at room temperature in dark for 95 min the absorbance of the mixture was read at 734 nm (A_{EXTR}), and ΔA was calculated as $A_{\text{ABTS}} - A_{\text{EXTR}}$. Trolox (Sigma Aldrich, USA) was used as a standard. Decrease in absorbance caused by trolox was done in the same way as for the samples, and standard curve $\Delta A/\text{trolox}$ concentration was created ($y = 496.11x - 18.506$, $R^2 = 0.9962$). Determination of antioxidant activity was done in three replications. Results were expressed in μmol of the trolox equivalents per litre.

D. Antioxidant Activity Determination (DPPH)

An aliquot of wine (50 μL) was mixed with 2 ml DPPH radical solution (0.1 mM in ethanol). The absorbance of the mixture was read at 517 nm during period of 30 min, results were expressed as the mean of three replications. Pure ethanol was used as a blank. Percentage of the inhibition was calculated according to (1):

$$\%inhibition = \left[\left(\frac{A_0 - A_t}{A_0} \right) \right] \times 100 \quad (1)$$

A_0 : absorbance of DPPH radical solution, A_t : absorbance after 30 min.

E. Colour Density Determination

Colour density was determined by Hanna Instruments HI 83742 instrument according to the instruction manual. Results were expressed as the mean of three repetitions.

F. Sensory Evaluation

Wines were evaluated according to the 100 points OIV official method [19], by the panel of 15 educated viticulture/oenology students. Students formed three evaluation commissions, which formed three repetitions. Results were expressed as the mean value.

G. Data Analysis

Chemical composition data were analysed by Statistica 13.1 software, using *post hoc* LSD at 95% level.

III. RESULTS AND DISCUSSION

Analysis of basic oenological parameters is presented in Table II. Although the amount of free and total SO_2 is not directly related to the wineification process because it was added just before bottling, it is very important to determine the values because of the method chosen for total polyphenols determination. Folin-Ciocalteu reagent reacts not only with polyphenols, but also with other compounds with high antioxidant activity, like ascorbic acid. Inorganic ions Fe^{2+} , Mn^{2+} , I^- and SO_3^{2-} also show reactivity toward this reagent [20]. Concentration of free SO_2 in samples is acceptable for this method. Alcohol content is high in all samples with no significant difference among wood and steel-fermented wines. Samples W3-W6 fermented in stainless steel tanks have similar level of total acidity. Sample W1 has higher level of total acidity probably due to the formation of small amounts of volatile acid, while sample W2 shows lower value of total acidity than other samples. Change in the level of acidity during fermentation is a consequence of fermentation abilities of certain yeasts and formation of aromatic esters, which in this case is favourable for authentic yeast strain. When comparing authentic and commercial yeast strain, it can be seen that both have good fermentation abilities. All tested wines reached dryness (less than 4.0 g/L residual sugar). Nevertheless, there is a small but significant difference in the sugar level between the authentic and commercial strain (Table III). The concentration of extract without sugar is very similar in all samples regardless the wineification technique, which is expected, since all wines are produced from the same starting grape.

TABLE II
BASIC CHEMICAL PARAMETERS IN TESTED SAMPLES ^{A, B}

Sample	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Alcohol (vol. %)	Total acid (g/L)	pH	Ash (g/L)
W1	5.57 ^a ±0.09	52.67 ^a ±0.45	14.83 ^a ±0.39	7.025 ^d ±0.03	3.66 ^a ±0.01	2.022 ^a ±0.04
W2	18.37 ^d ±0.27	87.36 ^b ±0.09	14.74 ^a ±0.00	4.825 ^a ±0.11	3.85 ^a ±0.00	2.055 ^a ±0.13
W3	8.45 ^b ±0.36	121.54 ^a ±0.27	14.69 ^a ±0.19	6.400 ^{bc} ±0.14	3.67 ^a ±0.00	2.497 ^c ±0.04
W4	16.70 ^c ±0.09	152.19 ^c ±1.45	14.60 ^a ±0.19	6.525 ^c ±0.03	3.50 ^a ±0.04	2.570 ^c ±0.02
W5	8.576 ^b ±0.18	119.04 ^c ±1.99	14.60 ^a ±0.06	6.300 ^b ±0.07	3.49 ^a ±0.01	2.032 ^a ±0.11
W6	21.952 ^c ±0.27	144.13 ^c ±3.26	14.47 ^a ±0.00	6.375 ^{bc} ±0.03	3.61 ^b ±0.00	2.280 ^b ±0.09

^A Results were expressed as the mean of two repetitions ± standard deviation.

^B Means followed by the same letter in the columns are not statistically different at 5% probability.

TABLE III
EXTRACT, SUGAR, POLYPHENOLS AND COLOUR IN TESTED SAMPLES ^{A, B}

Sample	Total extract (g/L)	Reducing sugar (g/L)	Extract without sugar (g/L)	Total polyphenols (g/L)	Colour density
W1	19.15 ^a ±0.21	0.33 ^a ±0.09	18.82 ^a ±0.31	0.423 ^a ±0.000	0.132 ^b ±0.002
W2	21.60 ^c ±0.00	3.72 ^d ±0.18	17.87 ^b ±0.18	0.557 ^d ±0.009	0.128 ^a ±0.001
W3	20.10 ^b ±0.00	1.51 ^b ±0.34	18.59 ^b ±0.34	0.487 ^b ±0.004	0.148 ^d ±0.000
W4	20.45 ^b ±0.21	2.00 ^{bc} ±0.56	18.45 ^{ab} ±0.06	0.507 ^c ±0.008	0.139 ^c ±0.001
W5	20.35 ^b ±0.78	1.76 ^{bc} ±0.33	18.59 ^b ±0.45	0.443 ^a ±0.004	0.149 ^d ±0.001
W6	20.90 ^{bc} ±0.00	2.11 ^c ±0.04	18.72 ^b ±0.06	0.454 ^a ±0.004	0.125 ^a ±0.001

^A Results were expressed as the mean of two repetitions ± standard deviation.

^B Means followed by the same letter in the columns are not statistically different at 5% probability.

Phenolic compounds are important components of wine. They contribute to their organoleptic characteristics (colour, flavour, astringency) and also act as antioxidants, with mechanisms involving both free radical scavenging and metal chelation [21]. The uncontrolled production of oxygen-derived free radicals is related to many diseases and degenerative processes: cancers, atherosclerosis, ageing. Living organisms developed different defence mechanisms to diminish the damage from reactive oxygen species like producing natural antioxidants or endogenous enzymes [22].

Wine phenolics belong to two main groups, non-flavonoid (namely hydroxybenzoic acid and hydroxycinnamic acid and their derivatives, stilbenes and phenolic alcohols) and flavonoid (namely, anthocyanins, flavan-3-ol monomers and polymers, flavonols and dihydroflavonols) [23]. Phenolic composition can be modified by yeast during fermentation, as a result of conversion of non-phenolic substances into phenolic or by solubilisation and extraction of phenolics by the ethanol. The contact of wine with wood also affects polyphenols profile [21]. The amount of phenols that migrate into wine depends on duration of ageing, the oak type, the seasoning of staves, the size of the barrel, the degree of oak toasting and previous usage of the barrel [24], [25]. Amount of total polyphenols in all tested samples (Table III.) is between 0.453 g/L (W1) and 0.557 g/L (W2). Typical values for white wines are usually smaller (100-300 mg/L) [26], but in wines which have not been aged in wood. Volatile phenols and phenolic aldehydes are formed by degradation of lignin, the furfural compounds formed by thermolysis of the cellulose and hemicellulose together with Maillard reactions which take place during barrels production [4]. Potentially extractable compounds present in the barrel wood depend on two main factors: geographical origin and processing of the wood [25]. In this case, the lowest level of polyphenols had samples W1, W5 and W6 which aged in Croatian barrels. Although for sample W6 aged two years in Slovenian barrels, in this case it did not show significant influence. On the other hand, sample W2 had significantly higher level of total polyphenols compared to the W1 sample. Since both of those samples fermented and aged by *sur lie* method in Croatian MT oak barrels, it can be concluded that the yeast strain had a main influence on such a result. Wine samples fermented in stainless steel and aged in French oak barrels (W3 and W4) had a significantly higher level of polyphenols than samples aged in Croatian oak. Contrary to expectations, low toasted oak acted favourably on the polyphenols content. Since polyphenols are not the only molecules with antioxidant activity in wine, high correlation between polyphenols content and antioxidant activity is not always the case, especially in white wines [27], [28]. Besides polyphenols, other molecules also show antioxidant properties like carotenoids or products of Maillard browning. Results for antioxidant activity strongly depend upon the method used for determination. Although both ABTS and DPPH methods are based on free-radical scavenging activity, results do not have to correlate [29]. In this research, correlation between the results obtained by ABTS and DPPH method is relatively high ($R^2=0.7778$), but

correlation between polyphenols and DPPH is much higher ($R^2=0.6503$) than correlation between polyphenols and ABTS ($R^2=0.3598$). Antioxidant activity depends not only on the phenolic concentration, but also on the specific chemical structure of each compound [28]. The lowest percentage of the inhibition of DPPH radical after 30 min of reaction showed W1 sample (51.73%), followed by sample W6 (54.20%) (Figs. 1 and 2), similar, as in the case of polyphenols. There is no statistically significant difference of the DPPH radical inhibition between samples aged in Croatian and French oak MT barrels (W5 and W3), while the LT barrel in a higher percentage of the inhibition. The highest antioxidant activity by both methods was shown as sample W2. Like in the case of polyphenols, different yeast strains affected the antioxidant properties of the wine more strongly than fermentation technique and barrel type.

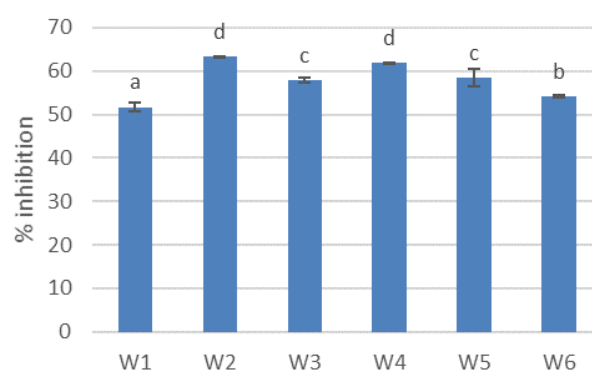


Fig. 1 Inhibition of DPPH radical after 30 minutes (Results were expressed as the mean of three repetitions \pm standard deviation; Means followed by the same letter are not statistically different at 5% probability)

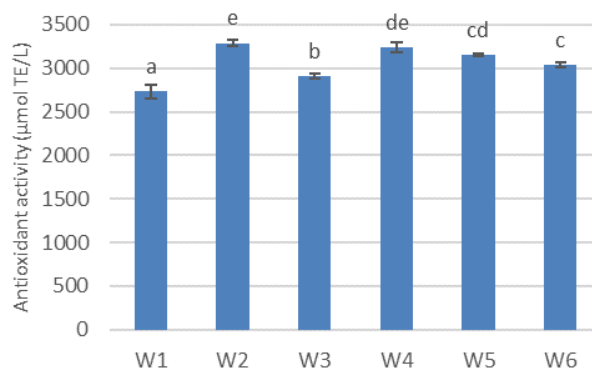


Fig. 2 Antioxidant activity in samples (ABTS) (Results were expressed as the mean of three repetitions \pm standard deviation; Means followed by the same letter are not statistically different at 5% probability)

Colour density is presented in Table III. The highest colour density had samples aged in new Croatian and French oak MT barrels, followed by new French LT barrel. Two year old barrels (W6) resulted in significantly lower colour density. Samples produced by *sur lie* method also had lower colour

density than samples produced by conventional fermentation. Oxygen permeation through wood favours redox processes and formation of new tannin derivatives and colour change [30]. In this case, colour does not depend only on amount of polyphenols but also on other compounds like products of browning reactions.

Sensory evaluation of tested samples is presented in Table IV. The order of tasting samples was done in a way to lower the impact of individual variation within a process: W1, W6, W5, W3, W4 and W2. Taking into account that the wines were prepared for the market, without any colour and clarity defects, the appearance of all samples was scored with the maximum number of points. The minimum score for odour was assigned to sample W1 (21.7 points out of maximum 30), while the odour of all other samples was scored similarly. Such a result indicates that French oak barrique barrels had an especially positive impact on the smell of the wine, regardless the level of toasting. The lowest grade for flavour was assigned also to the W1 sample. Comparing to other previously mentioned parameters (polyphenols, antioxidant activity, acidity), it can be concluded that ageing on commercial yeasts lees in Slovenian oak barrels negatively influenced the quality of Chardonnay wine. Still, 77 out of maximum 100 points for overall rate certainly cannot be considered as a bad score. According to Croatian legislation, sample W1, as well as sample W5, belong to the category of "quality wines with protected designation of origin" (category most represented in Croatian wine market). Ageing in new French oak LT barrel and in old Croatian barrel was scored with 35 out of 44 points for flavour, while new Croatian and French MT barrels achieved slightly lower results (33.7 and 32.0 points, respectively). The highest value of 38.0 points for taste (cleanliness, intensity, durability, quality) was assigned to sample W2. Fermentation in a new barrel with subsequent mixing of authentic yeast sediment positively influenced the quality of wine. Usage of Slovenian oak barrel for *sur lie* method of production and authentic yeasts resulted in excellent characteristics of wine, which was recognized by panellists (88 points out of 100 for overall rate) and also confirmed by chemical analysis. Beside sample W2, samples W3, W4 (French oak) and W6 (Croatian oak barrel two years old) also scored over 82 points for overall rate which places them in the category of "premium" wines (the highest category of quality) with especially pronounced Chardonnay wine characteristics.

TABLE IV

THE APPEARANCE (CLARITY, COLOUR), ODOUR (CLEANLINESS, INTENSITY, QUALITY), FLAVOUR (CLEANLINESS, INTENSITY, DURABILITY, QUALITY), HARMONY AND OVERALL RATE OF TESTED SAMPLES

	W1	W2	W3	W4	W5	W6
Appearance	15.0	15.0	15.0	15.0	15.0	15.0
Door	21.7	24.7	24.3	24.7	23.7	23.4
Flavour	31.3	38.0	33.7	35.0	32.0	35.7
Harmony	9.0	10.7	9.3	9.3	8.7	9.3
Overall rate	77.0	88.4	82.3	84.0	79.4	83.4

IV. CONCLUSION

All variations in wine producing, ageing in different oak barrels, *sur lie* and classical fermentation, had significant influence on all analyzed parameters. French oak barrels showed better results than Croatian oak barrels considering polyphenols and sensory evaluation. Low toasting affected positively the polyphenols content and antioxidant activity by both methods. Two year old Croatian barrels had low levels of polyphenols, antioxidant activity and colour density, but was scored as a premium quality wine by the panellists. The *sur lie* method needs further research especially because usage of authentic yeasts gave excellent results for both chemical and sensory properties.

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