Analysis of volatile aroma constituents of wine produced from Indian mango (*Mangifera indica* L.) by GC-MS

L. V. A. Reddy · Y. Sudheer Kumar · O. V. S. Reddy

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Abstract Volatile aroma compounds are synthesized by wine yeast during wine fermentation. In this study the volatile aroma composition of two varieties of mango wine were determined to differentiate and characterize the wines. The wine was produced from the fruits of two varieties of mango cultivars namely *Banginapalli* and *Alphonso*. The volatile compounds formed in mango wine were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). Thirty-two volatile compounds in wines were determined of which four were new and unidentified present in lower concentration. Apart from the ethanol (8.5 ± 0.28 and $7.2 \pm 0.28\%$ v/v), 1-propanol (54.11 ± 0.33 and 42.32 ± 0.57 mg/l), isobutyl alcohol (102 ± 1.57 and 115.14 ± 2.88 mg/l) and isoamyl alcohol (123 ± 2.88 and 108.40 ± 0.23 mg/l) were found to be the major flavouring higher alcohols in the mango wines produced from the fruits of *Banginapalli* and *Alphonso* respectively. Ethyl acetate (35 ± 0.57 and 30.42 ± 1.15 mg/l) was the major ester component in both wines produced. Besides, other esters like ethyl octanoate, ethyl hexanoate and ethyl decanoate were also present in the wines. Cyclohexane methanol (1.45 ± 0.11 mg/l) was present only in wine made from *Banginapalli* and β -phenylethyl butanoate (0.62 ± 0.01 mg/l) was found only in *Alphonso* wine. The results demonstrate that the wine prepared from *Banginapalli* variety had better aroma composition and good taste than that from the *Alphonso* variety.

Keywords Mango wine · Volatile constituents · GC-MS analysis

Introduction

Aroma profile is important in wine, as it contributes to the quality of the final product. It is due to the combined effects of several volatile compounds mainly alcohols, aldehydes, esters, acids, monoterpenes and other minor components already present in the grapes are being formed during the fermentation and maturation process [1]. Tropical fruits have been used as substrates for the production of wines [2–4]. In the tropics, fruits grow in abundance, even in the wild. One such fruit is the mango (*Mangifera indica* L.) which has the largest area under cultivation of any single fruit crop in the tropics [5]. Mango, the pride fruit of India, is an important tropical fruit crop occupying about 60% of the total area under cultivation in India. Twenty-five different mango cultivars are available in India, and are widely cultivated all over the world. It has a rich luscious, aromatic flavor and delicious taste in which sweetness and acidity are delightfully blended. It contains good amount of sugar (16–18% w/v) and many organic acids, and also a good antioxidant carotene (as vitamin A, 4,800 IU). Sucrose, glucose and fructose are the principal sugars in fully-ripened mango with small amounts of cellulose, hemicellulose and pectin [6]. The unripe fruit contains citric acid, malic acid, oxalic acid, succinic acid and other organic acids. In contrast, in ripe fruits, the main organic acid is malic acid [7]. Mangoes with higher initial concentration of β -carotene are also reported to be helpful as cancer preventing agents [8].

In European usage, wine and brandy refer exclusively to fermented byproducts of grape. In the new world,

L. V. A. Reddy¹ · Y. S. Kumar² · O. V. S. Reddy² (✉)

¹Department of Microbiology,
Yogi Vemana University, Kadapa – 516003, India

²Department of Biochemistry,
Sri Venkateswara University, Tirupati – 517502, India

E-mail: ovsreddy@yahoo.com

however, wines and brandies may refer to the fermented byproducts of any fleshy fruit or flower. For the first time Czyhrinciwk [9] reported the technology involved in mango wine production. Later several researchers [10–13] screened 20 varieties of mangoes that are available from India for wine production. According to their reports, the mango wine has similar characteristics to grape wine, but they have not given details on vinification technique and chemical composition of wine produced from mango. One of the methods of processing and preserving mango is to ferment the juice, which has high carbohydrate content, into wines. In keeping this view Obisanya et al. [14] studied the fermentation of mango juice into wine using locally isolated *Saccharomyces* and *Schizosaccharomyces* species from palm wine. Recently we [15] have screened 10 mango varieties available in India, and selected three varieties which yielded good quality wine. We also suggested that all the available mango varieties are not suitable for wine production and concluded that the *Banginapalli*, *Bangalore* and *Alphonso* varieties are most suitable for wine production. None of the above investigators have studied the composition of the volatile compounds of the mango wine. On the other hand, Pino et al. [16], Pino and Mesa [17] elucidated about 370 volatile components from 20 different mango varieties. As far as consumers are concerned, the aroma and flavor of wine are among the main characteristics that determine its quality and value [18, 19]. The aroma complexity dramatically increases during alcoholic fermentation as a result of the synthesis of important volatile compounds by the wine yeast and the release of some varietal aroma precursors [18]. The nature and amount of the synthesized volatile compounds depend on multiple factors, such as the nitrogen content of the must, the temperature of fermentation and the yeast strain [20, 21]. The volatile compounds synthesized by wine yeasts include higher alcohols (fusel, marzipan and floral aromas), medium and long-chain volatile acids (fatty, cheesy and sweaty aromas), acetate esters and ethyl esters (fruity and floral aromas) and aldehydes (buttery, fruity and nutty aromas), among others [22, 23]. The volatile fatty acids also contribute to the aroma of wines. Fatty acids are essential constituents of the plasma membrane and precursors of more complex molecules, such as phospholipids [20].

In the present investigation, characterization of volatile aroma nature of the wines produced from *Banginapalli* and *Alphonso* varieties were studied using gas chromatography coupled with mass spectrometry (GC-MS). This study will contribute a great deal towards a program aimed at the enhancement of the quality of the product derived from one of the India's most popular tropical fruits, the mango.

Materials and methods

Preparation of mango juice

Two varieties of ripened mango fruits (*Banginapalli* and *Alphonso*) were obtained from the local market of Tirupati (A.P.), India and stored at room temperature. The pulp was separated from the fruit by removing the peel and kernel. The pulp was blended in a waring blender and treated with 0.5% pectinase enzyme (Trizyme P50) procured from Triton Chemicals, Mysore, India, to increase the juice yield and kept at incubation for 3 h at 40°C. After incubation the juice is extracted from the enzyme treated mango pulp. Potassium metabisulphite 250 ppm was added as preservative and the juice was stored in the refrigerator at 4°C. The juice obtained in this manner was then subjected to analysis of free and total reducing sugars, free and total SO₂, total acidity, pH and soluble solids. None of the juices was ameliorated with sucrose.

Inoculum preparation

The wine yeast strain *Saccharomyces cerevisiae* 101 obtained from Central Food Technological Research Institute (CFTRI), Mysore was used in the experiments. The culture was maintained on MPYD agar (malt extract 3 g/l, peptone 5 g/l, yeast extract 3 g/l and dextrose 20 g/l, and agar 15 g/l) slants at 4°C. The yeast cells were activated by inoculating the slant culture into 25 ml of the sterile MPYD liquid medium in 100 ml Erlenmeyer conical flask, incubated on a rotary shaker (100 rpm) for 24 h at 25°C. The activated cells of 10% (v/v) containing 3×10^6 cells/ml were transferred into 250 ml conical flasks having 100 ml sterile mango juices (*Banginapalli* and *Aphonso*) for inoculum preparation and incubated for 24 h at 25°C.

Fermentation

A 250 ml portion of mango juice of *Banginapalli* and *Alphonso* varieties was transferred into two sterile 500 ml conical flasks and pH was adjusted to 4.5 using CaCO₃. Each flask is seeded with 10% (v/v) having 3×10^6 cells/ml of the 24 h yeast inoculum. The flasks were incubated at 20°C. Batch fermentation of the inoculated must was carried out over a period of 20 days. The specific gravity, sugar concentration, alcohol concentration, titratable acidity, pH and total soluble solids (TSS) were monitored during the fermentation. All the experiments were carried out in triplicate and mean values are presented in the paper. The samples were collected by separating the cells through centrifugation at 5,000 rpm for 10 min. The samples were

kept at -20°C for 2 weeks for chemical and sensory analyzes, and finally the wines were stabilized with an addition of 30 mg of SO_2/l . The wines were compared with sweet and dry table wines by a sensory evaluation. The wine color was analyzed by the spectrophotometric method [24], mainly to determine on a comparative basis the hue and brightness of the wines.

Sugar estimation

Sugar concentration was estimated by Shaffer and Somogyi [25] method. Total soluble solids were measured by estimating specific gravity of water soluble portion of the mash obtained by the centrifugation at 5,000 rpm for 15 min. The specific gravity was determined at 20°C with densitometer (Dynatrol, USA). With the aid of approximate tables, the results were converted to grams of soluble solids per 100 ml and expressed as grams of sucrose.

Ethanol and other volatile compounds

Ethanol and other metabolites (glycerol, methanol and total esters) were determined with the help of gas chromatography [26]. The fermented samples were centrifuged at 5,000 rpm for 10 min. The supernatant was used for ethanol analysis using an Agilent Systems Model 6890 plus instrument with the following conditions: 5% Carbowax 20 m glass column on Carbowax-B 80/120 mesh, with 2 m length, 2 mm inner diameter (ID), 1/4 mm. Nitrogen was used as a carrier gas with a flow of 20 ml/min and the eluted compounds were detected by flame ionization detector (FID). Fuel gas used in this process was hydrogen with a flow rate of 40 ml/min and the oxidant was air with a flow rate of 400 ml/min and n-propanol was used as an internal standard. Total acidity was determined by titrating with 0.1 N NaOH and the values were expressed as tartaric acid, and volatile acidity in the distillate samples is expressed as acetic acid mg/100 ml.

Analysis of volatile components by GC-MS

The analysis of volatile compounds was carried out by a Hewlett-Packard series 6890, gas chromatograph linked to an HP-5973 mass-selective detector equipped with fused silica capillary column (a $30\text{ m} \times 0.25\text{ mm ID}$, $0.25\text{ }\mu\text{m}$ film thickness HP-5MS, Agilent, Palo Alto, CA). The flow rate of carrier gas helium was 1 ml/min. The injection volume was $1\text{ }\mu\text{l}$. The injection temperature was programmed from 60°C for 2 min and then raised to 250°C at $4^{\circ}\text{C}/\text{min}$, held for 20 min. Injector temperature was maintained at 250°C . Mass spectra (MS) were acquired in the electronic impact

(EI) and positive chemical ionization (PCI) modes. The transfer line temperature was 250°C . MS were scanned at 70 eV electron impact mass spectrometry (EIMS) and 230 eV positive chemical ionization mass spectrometry (PCIMS) in the range m/z 29–350 atomic mass unit (amu) $1^{-\text{s}}$ intervals.

A sample of 100 ml of wine was adjusted to pH 7, by the addition of NaOH, and 1 ml of 4-methyl-2-pentanol (at a concentration of 10 mg/l) was added as an internal standard. The sample was extracted three times with diethyl ether. The sample was reduced to 1 ml by evaporating the ether solvent in a rotary evaporator at 40°C with low pressure. An aliquot ($1\text{ }\mu\text{l}$) of sample was injected into GC-MS. The identification of the volatile compounds was confirmed by comparing either their mass spectra (MS Chemstation Wiley 7N library) or with their retention times of standards. The analysis was carried out in triplicate.

Statistical analysis

Each experiment was repeated three times and results are expressed as mean $\pm$ standard deviation. The data was analyzed using SPSS statistical program (SPSS Inc., Chicago, IL) to determine the significant differences between the wines produced from *Baginapalli* and *Alphonso* varieties.

Sensory evaluation

The sensory analysis of various qualities attributes of wine samples (*Baginapalli* and *Alphonso*) were carried out as per the 9-point Hedonic scale with trained nine panelists in aspects of clarity, color, odor, taste, smoothness and overall acceptability [27]. The scores were used to evaluate the overall quality of wine.

Results and discussion

Fermentation

The mango musts after pectinase treatment have shown better fermentation performance compared to the controls without pectinase treatment. The juice yield was high from pectinase treated mango musts of *Baginapalli* ($550 \pm 5.77\text{ ml/kg}$) and *Alphonso* (570 ± 11.54). The incubation period for the fermentation was continued up to 20 days. The compositions of mango juice and wine are presented in Table 1.

The sugar concentration was determined in mango juice before fermentation. It was observed that $18.5 \pm 0.28\%$ in *Baginapalli* and $16.0 \pm 0.57\%$ (w/v) in *Alphonso*

Table 1 General composition of mango juice and wine

Juice composition	<i>Banginapalli</i>	<i>Alphonso</i>
Juice yield (ml/kg)	550 ± 5.77	570 ± 11.54
Total soluble solids	20 ± 2.88	16.5 ± 0.33
Residual sugars (% w/v)	18.5 ± 0.28*	16 ± 0.57*
Titrateable acidity (% v/v)	0.32 ± 0.02*	0.41 ± 0.02*
pH	4.0	4.2
Wine composition		
#Total acidity (% v/v)	0.60 ± 0.03**	0.80 ± 0.03**
†Volatile acidity (% v/v)	0.10 ± 0.03	0.20 ± 0.03
Ethanol (% v/v)	8.5 ± 0.28*	7.0 ± 0.28*
pH	4.0	3.8
Residual sugars (g/l)	1.5 ± 0.28	2.3 ± 0.17
Higher alcohols (mg/l)	343 ± 5.77**	300 ± 2.88**
Total esters (mg/l)	35 ± 2.88	25 ± 2.88
Total phenolics (mg/l)	610 ± 2.88***	725 ± 2.88***
Free SO ₂ (mg/l)	10	10
Bound SO ₂ (mg/l)	57 ± 0.57	58 ± 0.57

#As Tartaric acid; †As acetic acid; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

(Table 1). The principal metabolite produced by fermentation of the mango juice is ethanol. The presence of ethanol is essential to enhance the sensory attributes of other wine components. The concentration of alcohol affects the whole characteristic and taste of the produced wine. In the present study, the ethanol concentration from *Banginapalli* and *Alphonso* mango must was observed to be 8.5 ± 0.28 and $7.0 \pm 0.28\%$ (w/v) (Tables 1 and 2). The ethanol concentration of the wines, particularly from warm climates where grape sugar content is high, would reach above 15% (v/v) [28]. The results showed that the wine produced from mango must contained moderate ethyl alcohol concentrations like as in moderate grape wines.

The total acidity of mango wine samples ranged around 0.60 ± 0.03 and $0.80 \pm 0.03\%$ (w/v) and the volatile acidity was between 0.10 ± 0.03 and $0.20 \pm 0.03\%$ (w/v) and the pH of the finished mango wine was between 4.0 and 3.8 (Table 1). Wine contains a large number of organic acids. Among these predominant is tartaric acid and malic acid, which account for 90% titratable acidity. Wine acidity plays important role in maintaining the organoleptic properties of wine. The major acids affecting sourness in wine are tartaric, malic, and lactic acids. These acids can also induce astringency, presumably by denaturing saliva proteins [29]. The pH of the juice and wine has a profound influence on the survival and growth of all microorganisms [30].

Volatile constituents of wine

To know the volatile components and different types of esters and alcohols, the wine extract was analyzed by GC-MS. From this analysis nearly thirty two compounds were identified (Figs. 1 and 2). In this, isoamyl alcohol and esters were found to be major constituents (Table 2). The isoamyl alcohol and ester (ethyl acetate) were in comparable amounts to grape wine [31].

The higher alcohols produced by yeasts are the aliphatic alcohols such as 1-propanol, 2-methyl-1-propanol, amyl alcohols and the aromatic 2-phenylethanol. All higher alcohols produced mainly during the first stages of the fermentation, but 1-propanol forms throughout the fermentation reaching a maximum towards the end. 1-propanol have characteristic caramel, peach and sweet flavor, respectively [32]. In most cases, 2-phenylethanol forms early in the fermentation reaching a constant value at later stages and coming down towards the end of the fermentation. Isoamyl alcohol is the major higher alcohol found in wines (more than 50%) and its concentration has been reported in the range of 90–292 mg/l [33, 34]. Isoamyl alcohol (125.23 ± 2.88 mg/l and 108.40 ± 0.23 mg/l) was responsible for whiskey and malt odors, and isobutyl alcohol (102.40 ± 1.57 mg/l and 115.14 ± 2.88 mg/l) is responsible for bitter odor of wine. These were major part of higher alcohols in the wines of *Banginapalli* and *Alphonso* cultivars. Other higher alcohols like phenylethyl alcohol that contributes honey, rose and lilac odors and hexane-1-ol, which responsible for resin flavor and green grass cut odor, are the next major part of the mango wine volatiles. The cyclohexane methanol was not present in wine made from *Alphonso* variety mangoes, but in case of wine made from *Banginapalli* variety, it accounted for 1.45 ± 0.11 mg/l.

Esters are among the important groups of aroma compounds in wine. These are the fatty acid and acetate esters that are formed enzymatically during fermentation, which contribute to fruity and floral sensory properties to the wine [35]. Ethyl acetate is one of the important volatile compounds that present in wine and its presence will give the positive effect on the organoleptic characteristics of the wine. It contributes to increase in both the flavor and the taste of the wine [36, 37]. Among the six esters found in mango wine volatiles, ethyl acetate is responsible for pleasant pineapple odor in wine but contribute to defect at concentrations over 200 mg/l [38], which was detected in greater amounts (35.15 ± 0.57 mg/l) in *Banginapalli* wine than (30.42 ± 1.15 mg/l) in *Alphonso* wine. Other volatile esters like ethyl octanoate, ethyl decanoate, ethyl hexanoate and dimethyl styrene were observed in lower quantities of 1.15 ± 0.058 and 1.06 ± 0.01 mg/l, 2.34 ± 0.05 and 1.86 ± 0.05 mg/l, 0.942 ± 0.06 and 0.671 ± 0.01 mg/l, and $1.11 \pm$

Table 2 Volatile constituents in the wine produced from mango cultivars (*Banginapalli* and *Alphonso*) fermented at 20°C and pH 4.5 for 20 days

S. No.	Retention time (RT)	Name of the compound	Banginapalli (mg/l)	Alphonso (mg/l)
Alcohols				
1	1.271	Ethanol (%)	8.5 ± 0.28	7.2 ± 0.57
2	1.350	Ethyl ether	Solvent	Solvent
3	1.492	1-propanol	54.11 ± 0.33***	42.32 ± 0.57***
4	1.729	Isobutyl alcohol	102.40 ± 1.57*	115.14 ± 2.88*
5	2.581	Isoamylalcohol	125.23 ± 2.88**	108.40 ± 0.23**
6	4.823	2-furan methanol	0.223 ± 0.06	0.180 ± 0.01
7	6.535	Hexane-1-ol	1.42 ± 0.05*	1.02 ± 0.13*
8	12.900	Phenethyl alcohol	22.15 ± 1.15	24.15 ± 0.57
9	19.414	Cyclohexane methanol	1.45 ± 0.11	nd
10	42.58	n-pentane decanol	1.130 ± 0.05**	0.610 ± 0.05**
Esters				
11	1.665	Ethyl acetate	35.15 ± 0.57*	30.42 ± 1.15*
12	6.876	Ethyl hexanoate	0.942 ± 0.06*	0.671 ± 0.01*
13	15.92	Ethyl octanoate	1.15 ± 0.05	1.06 ± 0.01
14	20.124	Ethyl decanoate	2.34 ± 0.05**	1.86 ± 0.05**
15	33.62	β-phenylethyl butanoate	Nd	0.62 ± 0.01
16	19.67	Dimethyl styrene	1.11 ± 0.05*	1.34 ± 0.05*
Acids				
17	1.950	Acetic acid	0.201 ± 0.006*	0.163 ± 0.006*
18	3.292	Propanoic acid	0.145 ± 0.003***	0.217 ± 0.006***
19	3.829	Butanoic acid	0.932 ± 0.006***	0.745 ± 0.012***
20	12.655	2-furoic acid	0.910 ± 0.006***	0.548 ± 0.012***
21	15.482	Benzoic acid	1.08 ± 0.023	1.21 ± 0.058
22	15.750	Phenyl formic acid	0.643 ± 0.006***	0.912 ± 0.006***
23	16.723	Octanoic acid	0.735 ± 0.006***	0.427 ± 0.006***
24	37.99	Decanoic acid	1.180 ± 0.058*	0.963 ± 0.012*
Ketones				
25	2.850	Pentane-2 one	1.43 ± 0.115	1.15 ± 0.058
26	6.245	Furanone	1.12 ± 0.058**	1.51 ± 0.006**
27	11.489	Hydroxydimethylfuranone	0.238 ± 0.006***	0.452 ± 0.006***
28	25.967	2,6-bis(4-hydroxy-3-methoxy phenyl)	0.451 ± 0.006	0.432 ± 0.006
Unknown				
29	15.165	Unknown	0.183 ± 0.006***	0.412 ± 0.006***
30	23.377	Unknown	0.531 ± 0.006***	0.256 ± 0.006***
31	35.68	Unknown	0.441 ± 0.012***	0.131 ± 0.006***
32	38.86	Unknown	0.12 ± 0.012	tr

tr = component in trace level; Nd = not detected.

*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

Fig. 1 GC-MS chromatogram of the volatile compounds in *Banginapalli* mango wine.

Fig. 2 GC-MS chromatogram of the volatile compounds in *Alphonso* mango wine.

Table 3 Sensory evaluation of wine produced from *Banginapalli* and *Alphonso* varieties

Wine sample	Clarity	Color	Odor	Taste	Smoothness	Overall acceptance
Banginapalli	7.22	6.88	8.20	6.34	5.43	7.55
Alphonso	6.65	5.93	7.45	5.88	4.78	6.90

0.05 and 1.34 ± 0.05 mg/l in both *Banginapalli* and *Alphonso* mango wines, respectively. In general, these components have good characteristic aroma properties even in small quantities. β -phenylethyl butanoate (0.62 ± 0.01 mg/l) one of the important flavor ester was found in Alphonso wine but not in *Banginapalli* wine. Ethyl octanoate, ethyl decanoate, ethyl hexanoate and dimethyl styrene were responsible for apple, fruit fat and grape odor in wines [33]. Ester concentration and its relative distribution are governed by yeast strain [39] and fermentation conditions like temperature, pH, fatty acid/sterol levels and oxygen levels [31]. Therefore, many factors contribute to both the synthesis and hydrolysis of esters, and these factors differ in the time at which they may become significant during wine fermentation.

Acids such as benzoic acid (1.08 ± 0.023 and 1.43 ± 0.058 mg/l) and decanoic acid (1.18 ± 0.058 and 0.963 ± 0.012 mg/l) were found to be the major part of the volatile acids in the mango wine which influences sensory properties of wine. Apart from these two acids, propanoic acid and octanoic acids, which are responsible for pungent, rancid, soy, sweet cheese and fatty odors in wine, were present in lesser quantities. This may be due to the esterification of these acids with alcohols that result into esters.

The acetaldehyde content in wine produced from grapes is usually in the range of 13–30 mg/l [40]. In the present experiments, up to 30 mg/l acetaldehyde was found. Four ketone components, which are generally responsible for passion fruit, grape fruit odours were found in the mango wine. In these four, pentane-2 one (1.43 ± 0.115 and 1.15 ± 0.058 mg/l) and furanone (1.12 ± 0.058 and 1.51 ± 0.006 mg/l) were found to be the major constituents. Furanone with characteristic caramelized pineapple odor is found to be important in having organoleptic properties in many fruits [41].

From the statistical analysis, it was found that most of the volatile compounds were present in significant amounts in both wines. All these compounds impart characteristic aroma to the mango wine (Table 2).

Sensory evaluation results were presented in the Table 3. The overall acceptance of wines made from *Banginapalli* and *Alphonso* varieties were compared in terms of clarity, color, odor, taste and smoothness. From the above study it was observed that the wine produced from

Banginapalli was slightly better compared to wine produced from *Alphonso*.

Conclusion

In conclusion, the wines produced from two different mango varieties showed significant differences in aroma composition and intensity of sensorial attributes. The wine prepared from *Banginapalli* variety had better aroma composition than that from the *Alphonso* mango variety. Numerous other volatiles have odor activity values greater than one, and also may contribute to the unique wine flavor. The wines produced from both the varieties have characteristic aroma volatiles, which are usually found in grape wines. Further sensory studies to determine the volatiles that may truly define the mango wine aroma are being investigated by the authors. These studies further enhance the characterization and production of quality wines from other cheap varieties of mango fruits available in India. This study will contribute towards a program aimed at the enhancement of the quality and commercialization of the products derived from the mango.

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