Lignin-derived phenolic compounds in cachaça aged in new barrels made from two oak species

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ABSTRACT

Aging cachaça in wooden barrels is essential to improve its quality. The level of maturation of distillates can be determined based on the contents of aging-marker phenolic compounds extracted from the lignin of the wooden barrel. This study aimed to characterize the aging process of cachaça by analyzing the mechanism of lignin degradation during its maturation in new barrels made from two oak species, European (Quercus petraea) and American (Quercus alba), for up to 60 months. Evaluation was based on the analyses of cinnamic aldehydes (sinapaldehyde and coniferaldehyde), benzoic aldehydes (syringaldehyde and vanillin), and benzoic acids (syringic and vanillic acids) using high-performance liquid chromatography. Oak species had a significant effect on all the studied phenolic compounds. Higher contents of all the identified phenolic compounds were found in cachaça aged in barrels made from American oak. The total contents of benzoic acids (vanillic and syringic acids) can be considered for predicting the level of maturation of cachaça aged in barrels made from both oak species. Based on the composition of maturation-related congeners, it is likely that for cachaça each year of aging in new oak barrels corresponds to approximately 5 years of aging for spirits in general commercialized worldwide.

1. Introduction

Cachaça is the typical and exclusive designation of the sugar cane spirit produced in Brazil, containing 38–48% ethanol by volume at 20 °C, obtained by the distillation of fermented sugarcane juice (Bortoletto and Alcarde, 2013). Although aging is not mandatory for this distilled spirit, it is of vital importance to improve cachaça sensory profile. Brazilian law establishes that premium aged cachaça is the spirit matured in wooden barrels (maximum capacity of 700 L) for a period of not less than one year (Bortoletto and Alcarde, 2013).

The effects of the aging process are primarily influenced by the species used for making the barrel (Mosedale and Puech, 1998). Oak (genus Quercus) is the most suitable wood for aging beverages due to the aromatic complexity it confers to distilled spirits. European oak (Quercus petraea) and American oak (Quercus alba) are the most used species to make barrels for aging distillates (Chatonnet and Dubourdieu, 1998).

Low molecular weight phenolic compounds extracted from wood are incorporated into distillates during aging (Canas et al., 2008; Goldberg et al., 1999). Lignin transformations that take place during the aging process are among the most important factors that influence the quality of distilled beverages. The characterization of the level of aging of Scotch whisky, brandies, grape marc distillate, and wine distillates can be based on phenolic compounds derived from lignin (Aylott and MacKenzie, 2010; Cernişev, 2017; Rodríguez-Rodríguez and Gómez-Plaza, 2011; Rodríguez-Rodríguez and Gómez-Plaza, 2012; Rodríguez-Solana et al., 2014; Spillman et al., 2004).

Lignin macromolecules have branches of coniferyl (guaiacol compounds) and sinapyl (syringol compounds) alcohols (Köse and Gülçin, 2017; Gülçin et al., 2006). Coniferyl alcohol gives rise to coniferaldehyde, which is converted to vanillin and, in turn, oxidized to vanillic acid. Sinapyl alcohol generates sinapaldehyde, which is transformed into syringaldehyde and later oxidized to syringic acid (Puech, 1981) (Figure 1). Therefore, this study aimed to follow the evolution of lignin-derived phenolic compounds in cachaça during a 60-month aging process in new oak barrels made from two species of oak, European (Q. petraea) and American (Q. alba).

2. Materials and methods

2.1. Cachaça

The sugarcane spirit used in this study was produced in 2013 in the distillery of the Department of Agri-Food Industry, Foods, and Nutrition, Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo. The must was prepared using sugarcane variety SP 81-3250. Sugarcane juice was extracted using a stainless steel presser and its

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concentration was adjusted to 18°Bx using potable water for dilution. Fermentation was performed in 1,500-L tanks using Saccharomyces cerevisiae strain CA-11 (LNF Latino Americana, Bento Gonçalves, RS, Brazil) for 24 h at 30°C. Double distillation was carried out in a 1,000-L copper pot still (Alcarde et al., 2012). In the first distillation, alcohol was extracted from the wine until the distillate in the condenser outlet had approximately 1% ethanol (v/v), measured using an alcohol meter, resulting in a distillate fraction named phlegma. This fraction underwent

**Figure 1.** Transformations of lignin-derived aromatic compounds during the aging process of distilled spirits.

| Compound            | RT (min) | DL (mg/L) | QL (mg/L) | a     | b     | r²    |
|---------------------|----------|-----------|-----------|-------|-------|-------|
| Vanillic acid       | 24.02    | 0.05      | 0.16      | 1262.14 | 256.66 | 0.998 |
| Syringic acid       | 26.61    | 0.03      | 0.10      | 2427.27 | -103.23 | 0.998 |
| Vanillin            | 27.09    | 0.03      | 0.07      | 3111.94 | -86.94  | 0.999 |
| Syringaldehyde      | 29.17    | 0.05      | 0.17      | 1078.18 | 340.49  | 0.996 |
| Coniferaldehyde     | 34.81    | 0.02      | 0.07      | 4549.27 | 147.16  | 0.998 |
| Synapaldehyde       | 35.87    | 0.03      | 0.09      | 3216.12 | 101.88  | 0.995 |
the second distillation, resulting in the following fractions: “head” (1.5% of the boiler useful volume), “heart” (distillate fraction recovered after “head” and until the distillate fraction in the condenser outlet had 60% ethanol v/v) and “tail” (distillate fraction recovered after “heart” and until the distillate fraction in the condenser outlet had 1% ethanol v/v). The “heart” distillate fraction characterizes the Brazilian double-distilled sugarcane spirit, i.e. the cachaça.

2.2. Aging

Double-distilled cachaça (63.5% ethanol v/v) was aged at room temperature (22 ± 3 °C), 55 ± 10% relative humidity, and protected from vibrations, for 60 months, in triplicates, in new medium toasted (240 °C for 12 min) 225-L barrels made from two oak species, European and American (Tonelleries de Bourgogne, Meursault, France). Aliquots of 25 mL were collected at the same level from the center of the barrels at 3, 6, 12, 18, 24, 36, 48, and 60 months of aging for analyses of maturation-related congeners.

2.3. Analysis

Aging congeners were analysed using high-performance liquid chromatography (HPLC) in a Shimadzu equipment, model LC-10 AD (Tokyo, Japan), with two Shimadzu LC-20 AD pumps, an ultraviolet (UV)–visible detector Shimadzu SPD-20A, and an automated injection system (20 μL) with gradient elution (Bortoletto and Alcarde, 2013). The standards used in this study were vanillin, vanillic acid, syringaldehyde, sinapaldehyde, syringic acid, and coniferaldehyde, all from Sigma-Aldrich (St. Louis, MO, USA), purity >99%. Calibration curves were obtained for standard mixtures with contents ranging from 0.5 to 30.0 mg/L for phenolic aldehydes and from 0.5 to 20.0 mg/L for phenolic acids (Table 1). Analyses using HPLC had two mobile phases composed of water/acetic acid (98/2, v/v) and methanol/water/acetic acid (70/28/2, v/v/v) at a flow rate of 1.25 mL/min. A pre-column Shimadzu VP-ODS (1 cm × 4.6 μm) and a C18 reversed-phase column model Shimpack VP-ODS (4.6 mm, 25 cm × 5 μm) thermostabilized at 40 °C were used. The UV detector was programmed to operate at variable wavelengths. The samples were previously filtered using a Millex-HV filter with polyvinylidene difluoride membrane (13 mm diameter, 0.45 μm pore size).

Statistical analyses were carried out applying analysis of variance (ANOVA) and the Statistical Analysis System, version 9.3. The means of the results obtained from triplicate analyses were compared using the Tukey’s test (p < 0.05).

3. Results and discussion

3.1. Lignin-derived phenolic compounds

Both oak species conferred all the studied phenolic compounds to aged cachaça. The highest contents of all the identified phenolic compounds (cinnamic aldehydes, benzoic aldehydes, and benzoic acids) were found in cachaça aged in barrels made from American oak compared to those obtained for cachaça matured in European oak barrels. Syringaldehyde and benzoic acids were the main low molecular weight compounds in barrel-aged cachaça, regardless of the oak species used to produce the barrels.

The contents of cinnamic aldehydes (sinapaldehyde and coniferaldehyde) increased until 6 months of aging, and then decreased until 18 months, in cachaça matured in barrels made from both oak species (Figure 2A), probably due to oxidation to their respective benzoic aldehydes. From 18 months until the end of the aging time, the contents of cinnamic aldehydes remained steady for cachaça aged in barrels made from both oak species. Cachaça aged in American oak barrels exhibited higher contents of cinnamic aldehydes during the whole aging period, except for the samples analyzed at 60 months of aging.

In contrast, the contents of benzoic aldehydes (syringaldehyde and vanillin) increased until 48 months of aging, and then decreased until 60 months, in cachaça aged in barrels made from both oak species (Figure 2B), possibly because they were transformed into their respective benzoic acids. The contents of benzoic aldehydes in cachaça aged in American oak barrels were approximately twice as high as those found in cachaça aged in European oak barrels considering the whole aging period. In aged wine distillates, it has been shown that the contents of
Cinnamic aldehydes decrease, while the contents of benzoic aldehydes increase with aging time from 1 to 50 years (Cernîșev, 2017). The total contents of benzoic acids (syringic and vanillic acids) increased with the aging time (Figure 2C) in cachaça aged in barrels made from both oak species. Nevertheless, the increment in the contents of benzoic acids during the aging process of cachaça matured in American oak barrels was more pronounced.

Aromatic acids have been considered the main quality markers for aged wine distillates, and in those aged for more than 20 years, the contents of benzoic acids increased continuously, reaching 12.8 mg/L (Cernîșev, 2017). Similar values of total benzoic acids were found in cachaça aged in European oak barrels were detected in the samples aged for 60 months (16.18 mg/L). Cachaça matured in American oak barrels exhibited equal contents of both benzoic acids, whereas after 48 and 60 months, a prevalence of syringic acid (syringic acid/vanillic acid between 1.06 and 1.17) was registered (Figure 3).

The samples of cachaça aged in American oak barrels exhibited higher content of vanillic acid than that of syringic acid (syringic acid/vanillic acid increased from 0.26 to 0.77) up to 36 months. At 48 months, these samples exhibited equal contents of both benzoic acids, whereas after 48 and 60 months, a prevalence of syringic acid (syringic acid/vanillic acid between 1.06 and 1.17) was registered (Figure 3).

The ratio of syringic acid to vanillic acid increased with the aging time in cachaça aged in barrels made from both oak species (Figure 3). In cachaça matured in European oak barrels, the content of vanillic acid was higher than that of syringic acid (syringic acid/vanillic acid increased from 0.14 to 0.61) up to 24 months. At 36 months, these samples exhibited equal contents of both benzoic acids, whereas after 48 and 60 months, a prevalence of syringic acid (syringic acid/vanillic acid between 1.06 and 1.17) was registered (Figure 3).

Table 2. Content of lignin-derived compounds in cachaça aged for 60 months in new barrels made from two oak species, European (Quercus petraea) and American (Quercus alba). Errors represent standard deviation of independent triplicates.

| Aging period (months) | 3  | 6  | 12 | 18 | 24 | 36 | 48 | 60 |
|-----------------------|----|----|----|----|----|----|----|----|
| Guaiacol-type compounds | Q. petraea | 6.25 ± 0.19 | 14.06 ± 0.40 | 10.64 ± 0.36 | 11.53 ± 0.45 | 12.82 ± 0.52 | 13.77 ± 0.50 | 16.53 ± 0.60 |
| Q. alba | 13.79 ± 0.55 | 24.67 ± 0.92 | 19.89 ± 0.76 | 21.93 ± 0.90 | 23.31 ± 0.99 | 24.17 ± 0.97 | 26.72 ± 1.05 | 34.05 ± 1.42 |
| Sinapaldehyde | Q. petraea | 1.98 ± 0.10 | 12.55 ± 0.50 | 9.86 ± 0.44 | 2.12 ± 0.09 | 2.52 ± 0.10 | 2.37 ± 0.09 | 2.84 ± 0.11 |
| Q. alba | 3.00 ± 0.11 | 20.68 ± 0.86 | 14.00 ± 0.51 | 5.00 ± 0.21 | 4.47 ± 0.18 | 3.71 ± 0.12 | 3.97 ± 0.15 | 4.31 ± 0.17 |
| Syringaldehyde | Q. petraea | 4.02 ± 0.13 | 6.32 ± 0.21 | 7.28 ± 0.31 | 9.40 ± 0.37 | 11.03 ± 0.45 | 11.81 ± 0.43 | 17.98 ± 0.81 |
| Q. alba | 8.98 ± 0.34 | 11.68 ± 0.48 | 15.11 ± 0.71 | 18.45 ± 0.77 | 19.53 ± 0.91 | 20.95 ± 1.14 | 42.44 ± 1.88 | 37.76 ± 1.36 |
| Syringic acid | Q. petraea | 0.52 ± 0.01 | 1.84 ± 0.07 | 2.39 ± 0.09 | 3.34 ± 0.10 | 3.49 ± 0.10 | 6.33 ± 0.27 | 8.54 ± 0.35 |
| Q. alba | 2.10 ± 0.07 | 2.78 ± 0.09 | 3.98 ± 0.16 | 5.60 ± 0.18 | 6.98 ± 0.28 | 10.64 ± 0.37 | 16.84 ± 0.73 | 18.86 ± 0.81 |
| Syringol-type compounds | Q. petraea | 6.52 ± 0.24 | 20.71 ± 0.78 | 19.53 ± 0.84 | 14.86 ± 0.56 | 17.04 ± 0.65 | 20.51 ± 0.79 | 29.36 ± 1.27 |
| Q. alba | 14.08 ± 0.57 | 35.14 ± 1.43 | 33.09 ± 1.38 | 29.05 ± 1.16 | 30.98 ± 1.37 | 44.30 ± 1.63 | 63.25 ± 2.76 | 57.83 ± 2.21 |
The ratio of syringic acid to vanillic acid can be taken into consideration for characterizing the age of distillates. Cognacs aged for up to 2 years displayed prevalence of vanillic acid (syringic acid/vanillic acid from 0.67 to 0.89). Conversely, cognacs aged for 10–30 years exhibited higher content of syringic than vanillic acid (syringic acid/vanillic acid between 1.19 and 1.40) (Viriot et al., 1993). In grape marc distillates aged for 4 years in barrels made from European and American oak, this ratio was 1.50 and 1.48, respectively (Rodríguez-Solana et al., 2014).

The ratio of syringaldehyde to vanillin may also indicate the level of maturation of aged distillates (Gomez-Cordoves et al., 1997; Puech and Moutounet, 1992). In most distillates aged in oak barrels, this ratio is generally near 2, ranging from 1.4 to 2.5 (Canas et al., 2013; Canas et al., 2004; Cerniiev, 2017; MacNamara et al., 2001; Rodriguez-Solana et al., 2014; van Jaarsveld et al., 2009), denoting a possible correlation between the oxidation and transformation of these aldehydes. In this study, the ratio of syringaldehyde to vanillin ranged from 1.92 to 2.58 in cachaça aged in barrels made from European oak between 6 and 60 months, and from 2.38 to 4.00 in cachaça aged in American oak barrels during the whole aging time, pointing to the prevalence of compounds derived from sinapyl alcohol.

In addition, the relationship between benzoic aldehydes (vanillin and syringaldehyde) and cinnamic aldehydes (coniferaldehyde and sinapaldehyde) allowed the differentiation of the type of wood (chestnut or oak) used in the aging process of wine brandies (Canas et al., 2004). Orujo aged in American oak barrels had the highest ratio of benzoic aldehydes to cinnamic aldehydes (1.95), whereas in the beverage aged in European oak barrels this ratio was 0.79. Similar values were observed in orujo aged in barrels made from European oak of different origins, ranging from 0.84 to 1.07 (Rodríguez-Solana et al., 2014). In the present study, the relationship between these aldehyde families was higher for cachaça aged in American oak barrels, ranging from 6.49 to 12.93 after 36–60 months of aging, while in cachaça aged in barrels made from European oak, it ranged from 4.80 to 6.06 considering the same aging time.

### Figure 4

Total content of benzoic acids (syringic and vanillic acids) versus A: total content of guaiacol-type compounds (coniferaldehyde, vanillin and vanillic acid), versus B: total content of syringol-type compounds (sinapaldehyde, syringaldehyde and syringic acid) in cachaça aged for 60 months in new barrels made from two oak species, European (Quercus petraea) and American (Quercus alba). Error bars represent standard deviation of independent triplicates.

3.3. Guaiacol-type and syringol-type compounds

The highest contents of guaiacol-type and syringol-type compounds were found in cachaça aged in American oak barrels. The total contents of oxidation products deriving from coniferyl alcohol (guaiacol-type compounds) and sinapyl alcohol (syringol-type compounds) increased during aging (Table 2). However, the increment in the contents of these compounds was not constant during the aging period. For both oak species, the total contents of guaiacol-type compounds decreased from 6
to 12 months and then increased up to 60 months. In addition, the total contents of syringol-type compounds in cachaça aged in both oak species also decreased from 12 to 18 months and then increased up to 48 months, decreasing until the end of the aging period. The decrease in these aromatic compounds is due to chemical transformations of benzoic acids such as esterification, demethylation, decarboxylation, condensation, and conversion into volatile phenols, reactions that have also been reported for wine distillates aged for more than 20 years (Cernișev, 2017), or even because of other complex transformations of lignin-derived phenolic compounds (Viriot et al., 1993).

In wine distillates aged for up to 50 years, the total contents of guaiacol-type and syringol-type compounds increased during the maturation process, with higher contents of the latter (syringol-type/guaiacol-type compounds ranging from 1.60 to 2.09) (Cernișev, 2017). Similarly, the contents of syringol-type compounds were higher than those of guaiacol-type compounds in grape mark distillates aged for 5–6 years in European oak and in American oak barrels, with ratios of syringol-type to guaiacol-type compounds of 1.18 and 1.96, respectively (Rodríguez-Solana et al., 2014).

In this study, the composition of barrel-aged cachaça also showed prevalence of syringol-type compounds during the whole aging period, with the ratio of syringol-type to guaiacol-type compounds ranging from 1.33 to 1.84 for the samples aged in European oak barrels from 6 months on. Cachaça aged in barrels made from American oak exhibited ratios of syringol-type to guaiacol-type compounds ranging from 1.32 to 2.10 from 6 months of aging on.

The relationship between the total amount of benzoic acids (vanillic and syringic acids) and the total amount of guaiacol-type compounds and syringol-type compounds can be considered for predicting the level of maturation of cachaça aged in barrels made from both oak species.

![Graph](image)

**Figure 5.** Evolution of total contents of the analyzed phenolic aldehydes and acids during the 60-month aging process of cachaça in new barrels made from two oak species, European (Quercus petraea) and American (Quercus alba). Error bars represent standard deviation of independent triplicates.

### Table 3. Content of aging-marker compounds derived from lignin and some ratios among them in commercial aged spirits and in cachaça aged for 60 months in new barrels made from two oak species, European oak (Quercus petraea) and American oak (Quercus alba).

| Aging-marker compound | Content (mg/L) | Whisky (Aylott and Mackenzie, 2010) | Cognac (Viriot et al., 1993) | Wine distillate (Cernișev, 2017) | Cachaça aged for 5 years |
|-----------------------|----------------|-----------------------------------|-----------------------------|--------------------------------|-------------------------|
|                       | Aging period (years) | 12 | 10 | 20 | 30 | 10–20 | >20 | Q. petraea | Q. alba |
| Coniferaldehyde       |                | 3.9 | 2.4 | 1.8 | 2.4 |        |      |        |        |
| Vanillin              |                | 6.5 | 5.8 | 6.8 | 7.2 | 12.3   | 16.7 | 7.0    | 9.4    |
| Vanillic acid         |                | 3.6 | 3.1 | 4.0 | 5.4 | 3.5    | 4.5  | 7.8    | 22.2   |
| Guaiacol-type compounds |               | 19.7 | 23.6 | 16.6 | 34.0 |        |      |        |        |
| Vanillin/coniferaldehyde |         | 3.2 | 7.0 | 3.9 | 3.9 |        |      |        |        |
| Vanillic acid/vanillin |                | 0.6 | 0.5 | 0.6 | 0.8 | 0.3    | 0.3  | 1.1    | 2.4    |
| Sinapaldehyde         |                | 3.5 | 1.6 | 2.7 | 1.2 |        |      |        |        |
| Syringaldehyde        |                | 11.6 | 10.9 | 13.3 | 14.2 | 23.2   | 27.8 | 14.4   | 37.8   |
| Syringic acid         |                | 5.8 | 4.0 | 5.6 | 6.4 | 8.4    | 8.3  | 8.3    | 18.9   |
| Syringol-type compounds |            | 35.1 | 37.7 | 25.4 | 57.9 |        |      |        |        |
| Syringaldehyde/sinapaldehyde |     | 6.6 | 17.4 | 5.3 | 31.5 |        |      |        |        |
| Syringic acid/syringaldehyde |    | 0.5 | 0.4 | 0.4 | 0.5 | 0.4    | 0.3  | 0.6    | 0.5    |
| Syringaldehyde/vanillin |               | 1.8 | 1.9 | 2.0 | 2.0 | 1.9    | 1.7  | 2.1    | 4.0    |
| Benzoic acids         |                | 9.4 | 7.1 | 9.6 | 11.8 | 11.9   | 12.9 | 16.1   | 41.1   |
distillates can be distinguished from older ones based on this relationship. A good correlation was found between the level of maturation of barrel-aged cachaça and the contents of aromatic benzoic acids derived from lignin degradation in the present study, regardless of the oak species (Figure 4).

During the whole aging time, the total contents of aromatic compounds derived from lignin degradation found in cachaça aged in American oak barrels were approximately the double of those observed in cachaça aged in barrels made from European oak (Figure 5). Overall, the total contents of low molecular weight lignin-derived compounds stabilized or slightly decreased after 48 months of aging in cachaça matured in barrels made from both oak species. Further investigations are advisable to analyse the presence of high molecular weight lignin-derived compounds in barrel-aged cachaça. Our team has been conducting studies to quantify esters of benzoic acids in samples of cachaça aged up to 60 months using gas chromatography–mass spectrometry analyses.

3.4. Commercial aged spirits

The contents and ratios of lignin-derived aromatic compounds reported for commercial aged spirits such as Scotch whisky (Aylott and MacKenzie, 2010), cognac (Viriot et al., 1993), and wine distillates (Cernihev, 2010) are shown in Table 3 to establish a comparison with the present findings. The composition of maturation-related congeners observed in the oldest samples of cachaça obtained in this study (Table 2) is in line with the average profile of phenolic compounds found in whisky aged for 12 years (Aylott and MacKenzie, 2010) and cognac aged for 10 years (Viriot et al., 1993). Furthermore, the profile of maturation-related congeners registered in cachaça aged for 5 years in new oak barrels (Table 3) was similar to the average composition of phenolic compounds registered for old (aged for 10–20 years) (Cernihev, 2017) and extra-old (aged for more than 20 years) grape distillates (Viriot et al., 1993). Based on the composition of maturation-related congeners found in barrel-aged cachaça in the present study, each year of aging in new oak barrels corresponded to approximately 5 years of aging for spirits in general commercialized worldwide.

4. Conclusions

The results obtained in this study contribute to the knowledge of phenolic composition of cachaça aged in new oak barrels. Benzoic aldehydes (syringaldehyde and vanillin) and benzoic acids (syringic and vanillic acids) were the main low molecular weight compounds derived from the degradation of lignin found in barrel-aged cachaça. American oak barrels conferred higher contents of low molecular weight lignin-derived compounds to aged cachaça than European oak barrels. The level of maturation of barrel-aged cachaça can be characterized by evaluating the low molecular weight lignin-derived phenolic compounds present in the beverage. The total contents of benzoic acids (vanillic and syringic acids) can be considered for predicting the level of maturation of barrel-aged cachaça.

Declarations

Author contribution statement

M. C. Castro: Performed the experiments; Analyzed and interpreted the data.
A. M. Bortoletto: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
G. C. Silvello: Analyzed and interpreted the data.
A. R. Alcarde: Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data included in article supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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