## **Physical-Chemical Characterization of Fermented Coconut Water** (*Cocos nucifera L*)

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**Abstract**— This study was carried out with the objective aimed to characterize the physical and chemical composition of coconut water produced by 1 yeast strain selected from 3 strains isolated from farms that produces sugar cane spirit in the region of origin in the city of Salinas, MG. Physical and chemical analyzes were made, determining total acidity and alcohol content, acetic acid, higher alcohols, acetaldehyde, furfural, ethyl lactate, methanol and ethyl acetate. Due to the lack of studies on the fermented coconut water and being a new product, the test results were compared with the parameters found in the literature of wine and other fermented fruits, noting that the results are similar. The LBCM 678 strain showed the best performance among the others strains being selected for the final fermentation and to chemically characterize the fermented coconut water. The results were: Acetaldehyde  $0.23\pm0.015$  mg/100mL, Ethyl acetate-  $5.02\pm0.068$  mg/100 mL, methanol  $0.37\pm0.01$  mg/100mL, higher alcohols-  $4.64\pm0.046$  mg/100mL, furfural and ethyl lactate were not detected in the detection limits of the equipment, volatile acidity,  $-4.3\pm0.2$  mg/100mL and ethanol content-  $9.83\pm0.25$  °GL.

Keywords— selected yeasts; alcoholic fermentation; physicochemical analysis.

# Caracterização Físico-Química de Fermentado de Água-De-Coco (*Cocos nucifera L*)

**Resumo**— Este estudo foi realizado com o objetivo caracterizar a a composição físico-química do fermentado de água de coco produzido por 1 cepa de levedura selecionada dentre 3 cepas (LBCM 671, 676 e 678) isoladas de fazendas produtoras de cachaça na região da indicação de procedência da aguardente de cana do tipo cachaça de Salinas. Foram feitas análises físico-químicas, determinando acidez total e teor alcoólico, ácido acético, álcoois superiores, acetaldeído, furfural, lactato de etila, metanol e acetato de etila como parâmetros para seleção da melhor cepa. Devido à escassez de estudos sobre o fermentado de água de coco e por ser um produto novo, os resultados das análises foram comparados com os parâmetros encontrados na literatura sobre vinho e outros fermentados de mostos de frutas, notando que os resultados encontrados são similares. A cepa LBCM 678 foi a que apresentou melhor desempenho fermentativo dentre as demais sendo selecionada para a fermentação final e para se caracterizar quimicamente o fermentado de água de coco. Os resultados encontrados foram: acetaldeído-  $0,23\pm0,015$  mg/100mL, acetato e etila- $5,02\pm0,068$  mg/100mL, metanol-  $0,37\pm0,01$  mg/100mL, alcoois superiores-  $4,64\pm0,046$  mg/100mL, furfural e lactato de etila não foram detectados nos limites de detecção do equipamento, acidez volátil-  $4,3\pm0,2$ mg/100mL e teor alcoolico-  $9,83\pm0,25$  GL.

Palavras-Chave— leveduras selecionadas; fermentação alcoólica; análises físico-químicas.

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### I. INTRODUCTION

Coconut (Cocos nucifera) is a member of the family *Arecaceae* (palm family) and the only species of the genus Cocos. It is an agricultural and livelihood crop for many people in Southeast Asia, the Pacific region, Africa and some countries in Latin America. Often named 'tree of life' because of its versatility, coconut is a vital multipurpose crop grown throughout the tropical regions of the world. Coconut palms can grow in fragile environments and poor quality soil, where few alternative crops would thrive. Nearly one third of the world's population depend on coconut as their source of food and their economy (C. ODURO-YEBOAH et al., 2020).

In Brazil, coconut cultivation develops mainly along the coast, being found in areas from the state of *Pará to Espírito Santo* (FONTENELE, 2005). Current statistics show that Brazil has more than 280 thousand hectares implanted with the crop, practically in almost all the Units of the Federation (1.8 billion fruits) (IBGE, 2019).

The industrial use of the coconut fruit occurs through the processing of solid or albumen dried (copra) or fresh endosperm, the latter most used in Brazil, being used in the manufacture of products such as coconut milk and grated coconut , employed in the food industry of sweets, cakes, chocolates, chocolates, etc., or used "in natura" in domestic cooking. A more recent and rapidly expanding type of processing is the extraction and bottling of coconut water (liquid endosperm) through the application of processing and conservation technologies (PRADES, et al., 2012).

As it easily deteriorates once exposed to the air, most of the coconut water is consumed in its natural form in the areas where it is produced. Coconut water is commercially processed using ultra high temperature technology. However, it loses its delicate fresh flavor and some of its nutrients during heating (KAILAKU et al., 2017), so would be desirable a non-thermal process to protect the fresh flavor and nutrient content of coconut water (GAUTAM et al., 2017).

In general, coconut water is sold within the fruit itself, where it is sterile. Coconut water is the liquid from the endosperm found inside the coconut cavity that begins to form around 2 months after the natural opening of the inflorescence (LIMA, 2013). According to research, coconut water corresponds to 25% of the weight of the fruit, and its basic composition is 95.5% water, 4% carbohydrates, 0.1% fat, 0.02% calcium, 0.01% phosphorus, 0.5% iron, in addition to amino acids, vitamin C, B vitamins and minerals (KAILAKU et al., 2017). According to the Brazilian Legislation, in its Decree 6,871 of June 4, 2009, which regulates Law No. 8,918, of July 14, 1994, which provides for standardization, classification, registration, inspection, production and inspection of drinks, (article 49) Fermented with coconut water is the beverage with alcoholic content of four to fourteen percent by volume, at twenty degrees Celsius, obtained from the must of fermented coconut water (BRASIL, 2009).

The yeasts used in the manufacture of alcoholic beverages are generally strains of Saccharomyces cerevisiae. In spontaneous fermentations, a large number of species may be involved, with predominance of Saccharomyces cerevisiae. The fermentation process carried out with selected yeast strains has the advantages of favoring the faster start of the process, avoiding contamination risks presented by fermentation, faster and more uniform fermentation rates, greater yield and quality of the resulting product and eliminating variations in the bouquet of the drink (BARBOSA, 2013).

The chemical, physical-chemical and organoleptic characteristics of fermented beverages depend on several factors involved, especially in the fermentation process due to the metabolites produced by yeasts (GRANATO et al., 2014). The importance of metabolites, higher alcohols and esters mainly produced by yeasts during fermentation is reported by Souza et al., (2012) and Vidal et al., (2013). Most of the published works have focused on the use of selected *Saccharomyces cerevisiae* to produce fermented drinks (GONÇALVES, 2015).

Until now, there is few studies in which alternative forms of conservation or trans-formation are proposed. Consumers demand foods that, in addition to satisfying the taste, are healthy or contribute to their health, this demand can be covered by the so-called functional foods, hence the interest in the development of products through fermentation since these foods and Its component shave many possible health benefits (MELINI et al., 2019). Fermented foods can be produced with simple economic ingredients and techniques, and can contribute significantly to the human diet, especially in rural home sand communities in villages around the world.

This study was carried out with the objective aimed to characterize was to promote the fermentation of coconut water using 3 different strains of yeasts selected from 18 strains isolated from farms producing cachaça in the region of the indication of origin of cachaça cane spirit for the production of coconut water fermented and obtain the chemical characterization of the fermented coconut water produced using HPLC, GC-FID, GC-MS.

#### II. MATERIAL AND METHODS

Fermentations were carried out using the LBCM 671, 676 and 678 strains selected in a previous study (BARBOSA, 2013) belonging to the collection of the Microbiology Laboratory of the Federal Institute of Northern Minas Gerais-*Campus* Salinas.

Green coconuts were used, which were washed with neutral detergent and sanitized with chlorinated solution (100 mgL<sup>-1</sup>/15min/pH 6.8) (ROSA and ABREU, 2000). After sanitation, the coconuts were rinsed with good quality drinking water, containing an active chlorine concentration of around 0.5 mgL<sup>-1</sup>. The water was extracted, the Brix degree was measured and the amount of glucose to be added was calculated so that the must reached the total soluble solids concentration of 16° Brix measured with the aid of an Atago PAL 1 digital refractometer.

The strains of S. cerevisiae previously kept in YPD+20% glycerol at -80°C were reactivated and cultured in YPD medium (1% yeast extract, 2% peptone, and 2% glucose). The inoculants were obtained according to Duarte et al., (2010). After reactivation in 1mL of YPD, the inoculums were transferred to increasing volumes up to 1 L. From this point, every 24 h, the cells obtained were removed from the flask in sterile water and stored at 4°C; a small amount (10%) was left in the flask and fed back with sterile YPD broth. This process was repeated for 3 days until sufficient cells were obtained for the inoculum. Viability and population counts of yeasts were determined using Neubauer Chamber by staining with methylene blue and microscopic analysis. The inoculum composed of 4.92x10<sup>8</sup> cells/mL of strain 671, 1.40x10<sup>8</sup> cells/mL of strain 676 and 2.4x108 cells/mL of strain 678 were used to ferment batches of 1 L of coconut water at 16 ° Brix. autoclaved (121°C, 20 min). The pH of the wort was measured at 25 ° C, using a pot of the brand Digimed, model DM-20, obtaining the value of 5.5. To avoid cell stress of yeast strains, fermentation was carried out using the fed batch method. The fermentation process was started using 0.4 L of sterile coconut water for each inoculum. After that, the Brix degree was monitored every 1 h and, when the must reached 3°Brix, 0.15 L of sterilized coconut water was added. This procedure was repeated 4 times until the volume of the vats was completed to 1 L. This point was considered as the initial fermentation time (T0). During the fermentation process, 3 consecutive triplicate fermentations were carried out for each strain and the vats were kept in a shaker incubator at 28°C until the Brix degree value stabilized in the interval between 0.0-0.5, which was defined as the standard to determine the end of fermentation after approximately 24 h (T24). The useful

The parameters described below were analyzed in the fermented coconut water in order to chemically characterize the produced drink. For the determination of the alcoholic degree of the fermented (% v/v), at 20°C and the acidity in mg/100 mL, an electronic distiller (Super DDE by Gibertini) was used. The alcoholic degree was determined based on the alcohol separation of the sample, by distillation, and its subsequent measurement on the electronic densimeter (ANTON PAAR brand model DMA 35A). And acidity by titrometry, with 0.01 mol/L sodium hydroxide solution, according to the standards of ABNT (1997), Brazil 2005. All samples were analyzed in duplicate. Ethanol and acetic acid were determined in the fermented by HPLC. Before HPLC and GC-FID analyzes, the samples were filtered through a milipore filter with an opening of 0.22 µm. The main major volatile compounds were analyzed by GC-FID according to Duarte et al. (2011). The determination of acetaldehyde, ethyl acetate, methanol, furfural and higher alcohols were performed by gas chromatography, Agilent gas chromatograph, with flame ionization detector, using a 60 m, 0.25 capillary polyethylene glycol (Supelco) column mm internal diameter and 0.25 µm film thickness. The chromatographic conditions were as follows: injector temperature equal to 225°C, detector temperature equal to 280°C; flow of carrier gas (helium) 30 mL/min .; flow of gases in the detector, hydrogen 30 mL/min. and synthetic air 300 mL/min. and split rate equal to 30. The temperature programming of the column oven, during the chromatographic analysis, was initially at 50 ° C for 6 minutes and then was raised to 100°C, at a ramp of 15° C/min., up to 190°C (standards) and 250°C (samples) for 2 min., at a ramp of 20°C/min. The injected volume was 2.0 µL of the distillate. The injections were performed in triplicate (SOUZA, 2010). То perform the chromatographic analyzes it was necessary to perform a standard curve, using the patterns of the investigated substances, in different concentrations. The following standards (Merck) were used: isoamyl acetate (HPLC grade), isoamyl alcohol (PA grade), ethanol (PA grade), ethyl acetate (PA grade), methanol (PA grade), 1-propanol (PA grade)), acetaldehyde (grade PA), isobutanol (grade PA) and furfural (grade PA). Such standards were prepared in 40% v/v alcoholic solution (HPLC-Fisher Scientific grade ethyl alcohol and deionized distilled water. Using the peak areas of these standards associated with the peak areas of the internal standard used (n-pentanol-2.5%), an index was obtained associated with the standard concentration used, based on these two parameters

(standard concentration x standard substance area/internal standard area ratio), the linear regression equation of the graph was obtained and the quantity of each compound of each sample was determined.

#### III. RESULTS AND DISCUSSION

For all strains tested, the same fermentation conditions were adopted. The 3 strains studied showed the same behavior for the consumption of soluble solids, per hour, because the higher the consumption, the better the performance of the yeast.

The strains showed a high consumption of soluble solids, thus showing a high fermentative performance. The strains were subjected to 10 days of conducting the experiment, where the strains had 10 fermentative cycles.



Fig.1 - Evaluation of the consumption of soluble solids. Average consumption of soluble solids

The alcoholic levels and the concentration of acetic acid obtained for the fermented are shown in Figure 2. These criteria are directly linked to the production yield. The strains showed excellent performance, but did not differ. The alcoholic levels of the samples showed average values between 8.10 and 9.20% v/v, which can increase in systems with greater efficiency and fermentation control (BARBOSA, 2013). Yeast ethanol production is usually in the range of 5% to 10% v/v (LIMA, 2013), whose variation in concentration was observed in the present study as well as in other works found in literature. Barbosa (2013) carried out 15 fermentations of sugarcane juice, using selected yeasts and found values of alcohol content ranging between 7.25 and 8.9% v/v. The averages of ethanol production at the end of fermentation showed significant differences (p<0.05) only for the LBCM 678 strain in relation to the LBCM 671 and 676 strains, which did not differ between them.

Liang et al. (2020) studied three different varieties of Hong Qu wine fermentation starters, and found significant differences in alcohol content. Some organic acids excreted in the fermentation medium are derived from intermediate pathways, such as acetic, malic and succinic (TAVARES, 2009). Acetic acid, which is the organic acid predominantly excreted in the growth medium, is produced by oxidation of acetaldehyde, with removal of hydrogen, in the reaction opposite to the normal reduction of acetaldehyde to ethanol (JANZANTTI, 2004).

Acetic acid is quantitatively predominant and its concentration varies from 60% to 95% of the total acidity. The excess of acidity promotes an undesired and slightly "aggressive" flavor in sugarcane spirit, depreciating the quality of the DRINK (PINHEIRO, 2010). The proportions of acids in alcoholic beverages are largely determined by the yeast lineage and fermentation conditions and, to a lesser extent, by the substrate used (MISHINA, GOMES, MORAIS, 2016). *S. cerevisae* in the presence of oxygen can convert up to 30% of the wort sugar into acetic acid (RAMOS, 2015).

Regarding the total acidity of the fermented, the 3 strains studied did not differ statistically from each other,

thus showing the same behavior in the fermentation process. As can be seen in Figure 2. According to data in the literature, there is a positive correlation between the wine's increasing acidity and the concentration of bacteria in the must, just as there is a negative correlation of these with alcoholic yield.



Fig.2 - Evaluation of the alcoholic content and acidity of the fermented. Average alcohol content of the fermentate expressed in % (v/v) and total acidity of the wine expressed in g of acetic acid/100mL of the fermented.

Acetaldehyde is a compound that reduces the quality of beverages and, when ingested, interferes with the central nervous system, so it is important to quantify it, and its concentration should be kept to a minimum. Aldehydes are common in the initial fermentation process, tending to disappear at the end. The cause of the excess of aldehydes in the drinks can be an indication of spontaneous oxidation (ROSCA et al., 2016).

Volatile esters are responsible for the fruity aroma of fermented drinks and consequently constitute a vital group of desirable aromatic compounds in beers and wines (VIEIRA, 2016).

 Table 1- Volatile compounds (mg/100 mL of anhydrous alcohol) in fermented products obtained by the 3 different yeast strains produced on a laboratory scale (1 liter).

Strain	Acetaldehyde	Ethyl Acetate	Methanol	Superior Alcohols	Furfural	Ethyl Lactate
671(mg/100mL)	2,2	4,10	nd	29,6	nd	nd
676(mg/100mL)	1,8	3,80	nd	32,75	nd	nd
678(mg/100mL)	1,2	4,60	nd	37,38	nd	nd

nd - not detected within the device's detection limit

Higher alcohols and esters are compounds present in fermented drinks that are incorporated into the flavor and aroma of the drink. These compounds are present in small amounts, but enough to offer characteristics specific to the fermented drink (SILVA, 2016).

The reduction in the acid content is relevant due to its effect on the acidity of the drink. According to Cisilotto

(2017), acidity can significantly and negatively influence the sensory quality of fermented drinks.

Most esters are made up of ethyl esters, formed during fermentation and distilled along with ethanol. These reactions occur because ethanol can react with acids derived from pyruvic acid, such as lactic and acetic acid, as well as short chain organic acids (butyric, capric, caprylic, capric and lauric) (MISHINA, GOMES, MORAIS, 2016). Several factors interfere in the synthesis of esters, such as the yeast strain, the composition of the medium, aeration (inhibits the formation of esters) and temperature. The main ester of cachaça is ethyl acetate, which in small concentrations incorporates a fruit aroma into the drink, which is desirable and pleasant. In excessive amounts, however, it gives an undesirable and sickening aroma (RIBEIRO, 2016).

Regarding the esters, an average content of the reference compound (ethyl acetate) of 4.4 mg.100mL<sup>-1</sup> was found (SOUZA et al., 2012).

As for total upper alcohols, the concentration range ranged from 27.16 to 37.38 mg.100mL<sup>-1</sup>.

Regarding methanol levels (mg100 mL<sup>-1</sup>), they were not detected within the detection limit of the device.

Aromatic compounds play an important role in the aroma and flavor of alcoholic beverages derived from fermentations performed by yeasts, thus contributing to the acceptance or not of the product by the consumer market (SOUZA, 2010). Thus, the 3 strains selected for the production of fermented coconut water, lines 671, 676 and 678, were evaluated for possible differences in the production of n-propanol, isobutyl alcohol and isoamyl alcohol, as well as the production of acetate esters. ethyl, isoamyl acetate, ethyl hexanoate, ethyl octanoate and ethyl decanoate. As can be seen, the 3 strains had the same formation profile.

Volatile esters are responsible for the fruity aroma of fermented drinks and consequently constitute a vital group of desirable aromatic compounds in beers and wines. The ethyl acetate analyzed showed a difference in the fermentative kinetics of strain 678.

In the laboratory scale fermentation study, of the lines analyzed LBCM: 671, 676, 678, the line LBCM 678 showed better fermentative performance and physicalchemical parameters according to the discussion above and with the analyzes made and presented in Figure 3.



Fig.3 - Evaluation of fermentative parameters

Thus, the LBCM 678 strain was used to chemically characterize the fermented coconut water. Samples from a triplicate fermentation were analyzed. The results are shown in Table 2.

Table 2 - Volatile compounds (mg/100 mL anhydrous alcohol) in the fermentation obtained by strain LBCM 678.

Strain	Acetaldehyde (mg/ 100mL)	Ethyl Acetate (mg/ 100mL)	Methanol (mg/ 100mL)	Higher alcohols (mg/ 100 mL)	Furfural (mg/ 100mL)	Ethyl Lactate (mg/ 100mL)	Acidity g acetic (acetic/ 100mL) ferm.	Alcohol content (%v/v)
LBCM 678	0,23±0,01	5,0±0,06	0,37±0,01	4,64±0,04	nd	nd	4,3±0,2	9,80±0,25

nd - not detected within the device's detection limit

The analysis of volatile components (acetaldehyde, ethyl acetate), methanol, ethanol, higher alcohols (1-propanol, isobutanol, amyl and isoamyl) and total acidity are parameters to check if the product's characteristics are within the limits established by legislation Brazilian.

According to the physical-chemical analyzes it was observed that compounds that depreciate the quality of the fermentate such as acetaldehyde, furfural, methanol and ethyl lactate were well below the limits established by Brazilian legislation. While the compounds that show the quality, such as ethyl acetate and higher alcohols, showed high values in comparison with other fermented fruits, however within the limits of the current legislation (BRASIL, 2005).

As for the content of ethyl alcohol ( $9.80^{\circ}$ GL), expressed in% of ethanol in volume at 20 ° C, it appears that the fermented produced is within the limits established by the Brazilian legislation on drinks, that is, Article 72 of the Section 2 of Decree No. 2,314, of September 4, 1997 and for Brazil (2005).

The high production of total acidity gives the product an unpleasant vinegar taste. The total acidity of the wine must be in the range of 3.3 to 7.8 g/L (TORRES NETO et al., 2006). It was found that the fermented had a concentration of 4.30 g/L. This value is similar to that reported by Duarte et al., (2011), endorsing the greater capacity of acetic acid production by pure S. cerevisiae. The reduction in the acetic acid content is relevant due to its effect on the acidity of the drink.

In the beverage industry, it is important to know the concentrations of acetaldehyde, as it has a central role in the manifestation of alcohol intoxication. Brazilian law allows the following maximum values of total aldehydes in distilled beverages, expressed in acetaldehyde/100 mL of anhydrous ethanol: brandy 30.0 mg/100 mL, cognac 40.0 mg/100 mL, rasp 80.0 mg/100 mL, simple grape distillate 40.0 g/100 mL, pisco 200.0 mg/100 mL (BRASIL, 2005).

Generally, the cachaça produced by selected yeasts showed higher concentrations of desirable volatile compounds. The main volatile compounds were identified by GC-FID (Table 2).

Concentrations between 50 to 80 mg/L contribute to the product's aroma (RIBEIRO, 2014). Ethyl acetate was found in the fermented coconut water at a concentration of 50.00 mg/L, which provides a positive impact on the quality of the drink (SOUZA et al., 2012). This result is in line with those reported by Erten and Tanguler (2010), which shows the ability of yeasts to improve the production of ethyl acetate. The high concentration of ethyl acetate found in fermentates produced by inoculation of selected yeasts, probably occurs because of its  $\beta$ -glycosidase activity (GAENSLY, 2016).

The presence of higher alcohols is associated with "alcoholic aroma" and its production by different strains of S. cerevisiae was reported by Araújo (2018). These higher alcohols are fundamental to the taste and aroma of the drink.

The maximum concentration established by Brazilian legislation for higher alcohols is 4500 mg/L of anhydrous alcohol (LEITE et al., 2013). Table 2 shows that the maximum was 46.40 mg/L. Thus, the product did not exceed the maximum allowed limit for the sum of the higher alcohols in the fermented product.

Acetaldehyde represents about 90% of the total concentration of wine aldehydes and wine distillates and is a product of the oxidation of ethanol (CORRÊA, 2015). It can be seen that the fermented coconut water has a concentration of  $2.30 \pm 0.01$  mg/L of acetaldehyde. White wines or similar products, such as the fermented coconut water, which have a concentration above 700 mg/L of anhydrous alcohol indicate that the fermented wine was subjected to aeration (aerated or oxidized) or to high doses of sulfitation of the must before alcoholic fermentation. The results show that the fermented coconut water has a concentration much lower than 700 mg/L of acetaldehyde, since it has not undergone any treatment such as aeration and sulfitation of the must.

Among the analyzed constituents, methanol is emphasized as one of the most important, as its production is undesirable and if it occurs, it should not exceed the limit of 35 mg/100 mL of the drink or 500 mg of methanol/100 mL of anhydrous alcohol (OLIVEIRA, 2016). As can be seen in Table 2, the methanol value was lower than the maximum allowed, which demonstrates that the removal of pectin from the must was quite efficient, because in the alcoholic fermentation process, methanol is formed by the degradation of pectin, a polysaccharide present in coconut water (ALVES, 2017).

Furanic aldehydes, furfural (F) and 5hydroxymethyl-furfural (HMF) are compounds formed during non-enzymatic browning reactions in food. The toxicity of these furan compounds in humans is not well known, with the exception of furfural, for which the IDLH ("Imediately Dangerous to Life or Health Air Concentration") value, which is 100.00 ppm, is already established. The literature suggests that prolonged or repetitive contact with furfural may cause dermatitis, irritation of the mucosa and respiratory tract, in addition to affecting the central nervous system. Both (F) and (HMF) can be considered indicators of food and beverage degradation, when found in large concentrations (WEBER, 2017). Furfural was not detected within the device's detection limit in coconut water fermentation.

The physical-chemical analyzes showed that the fermented coconut water has qualities comparable to other fermented fruits, such as cashew, orange, and wines produced from grapes.

#### IV. CONCLUSIONS

The S. Cerevisiae LBCM 678 strain positively influenced the final quality of the fermented coconut water producing: higher levels of desirable compounds, such as esters and higher alcohols, lower volatile acidity, higher concentration of ethanol and lower concentration of acetaldehyde and methanol.

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