

The effect of chemical treatments on the pH & microbial flora of cassava residues during storage

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Abstract

Cassava starch factories produce residues that can be commercialized as food ingredients. The objective of this study was to evaluate the microbiological safety of cassava peel and bagasse during storage, with and without chemical treatment. The bagasse was acidified with lactic acid, and the peel was immersed in a sodium hypochlorite solution. The microbiological analyses were carried out for 72 h after harvest. All of the samples showed the absence of pathogenic microorganisms, and the acidification and sanitization were effective in controlling total coliforms. Cassava bagasse and peel samples can be considered safe for consumption by humans as ingredients for other food products.

Keywords: *Manihot esculenta* Crantz; microbiology; residues.

1 Introduction

Cassava (*Manihot esculenta* Crantz) is grown in more than 90 countries, with an overall production of 242,000,000 t of roots in 2009. Among the continents, Africa is the main producer (50.7%), followed by Asia (33.8%) and South America (14.9%) (FOOD..., 2010). In 2010, Brazil produced 26,595,000 t of cassava, representing 11% of the world production (GROXKO, 2010).

Cassava roots are used directly or indirectly in culinary preparations in the form of flour and starch (CEREDA, 2001). In 2009, Brazil produced 583,850 t of cassava starch (ASSOCIAÇÃO..., 2010), and an estimated million jobs were generated in the phases of primary production and subsequent processing of the flour and starch. The parts of the root that are transformed into flour and starch produce revenues equivalent to 600 million dollars and 150 million dollars, respectively (SOUZA; FIALHO, 2003).

In the starch factories, for each ton of roots processed, about 250 kg of starch, 928.6 kg of bagasse with 85% moisture content and 428 kg of peel with 80% moisture content, are produced (LEONEL, 2003). During harvest, this excessive production of residues is considered a problem due to the need to transport and storage and their inadequate disposal. In addition to polluting the environment, it represents a loss of yield to the industry since cassava bagasse and peel contain mainly moisture, starch and fiber (CEREDA, 1994).

If the cassava residues were used as human food, even if the roots were washed, some soil particles would remain adhered to them, which could compromise food safety. Microbiological contamination of agricultural products can occur at all stages,

from the harvest period up to the processing, packaging, transport, and storage steps, and it can arise from the soil, water or air as a result of physical, mechanical, or manual contact. However, microbiological growth depends on the substrate, that is, on the biological conditions the food offers, which are mainly related to the availability of the water necessary for the metabolic processes of the microorganisms (FERREIRA NETO et al., 2004).

In order to use the solid residues from the processing of cassava starch for human consumption, for example in the form of flours, it is important to know the microbiological behavior of the product during in natura storage at room temperature since the residues would have to be stored for a certain period until there was sufficient amount to process.

Treatments such as adding acid to reduce the pH and reduction of the microbial load by adding a solution of disinfectant can modify the conditions necessary for microbial multiplication (FRANCO; LANDGRAF, 2005), and such operations may be strategic for maintaining the quality of cassava peel and bagasse during the initial storage of the product preceding drying.

The Brazilian cassava starch industry is interested in exploiting these 'new' raw materials, formerly designated as residues, which could be made available on the human food market. The use of the Flash Dryer of the cassava starch factory itself is suggested for this purpose by first reducing the moisture content of the residues to 30% using decanters or centrifuges and then drying them to 14%.

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Thus, the residues would be accumulated during part of the day, while the dryer was being used to dry the cassava starch, to be dried at a different time.

Time intervals would be established for each product as a function of their generation and the capacity of the dryer, thus avoiding new investment by the industry, or even, if the equipment allowed, adjusting the capacity to economize energy. Therefore, the objective of this study was to evaluate the microbiological profile of cassava peel and bagasse during room temperature storage, with and without chemical treatment, involving sanitization with sodium hypochlorite and the addition of lactic acid, respectively, focusing on exploiting these solid residues for human consumption.

2 Materials and methods

2.1 Material

The cassava bagasse and peel samples of the IAC-12 cultivar were donated by the *Fecularia Bela Vista Ltda.* (cassava starch factory), located in the town of *Bela Vista de Goiás*, State of Goiás, in the central region of Brazil.

2.2 Sampling

Triplicate samples were collected from the exit of the pipe that transports the residues to the storage unit, at 30-day intervals from September to November of 2010. They were packed into Bioline® low density polyethylene bags and immediately transported to the laboratory in isothermal boxes containing mineral water ice to maintain the temperature lower than 4 °C. In order to make the microbiological analyses of the residues feasible, 700 g of each sample were separated and packed into 7 identified sterile plastic bags (100 g each). These bags were maintained at room temperature considering the following pre-defined time intervals: zero, 3, 6, 12, 24, 48, and 72 h after collection. The microbiological analyses were carried out on the samples before and after chemical treatment.

2.3 Chemical treatment

Part of the cassava bagasse was submitted to acidification, spraying with sufficient 0.12M lactic acid (Sinth®) to reach pH 4.5, using a sanitized manual plastic sprayer. The other part of the cassava peel was sanitized by immersion in a 200 ppm sodium hypochlorite solution (Cromoline®) for 15 minutes.

2.4 Microbiological and pH analyses

The following microbiological analyses were carried out: yeast and mold count, total and heat-resistant (45 °C) coliform counts, counts of *Bacillus cereus* and sulfite-reducing Clostridia, and a search for the presence of *Salmonella* sp. using the techniques described by the *American Public Health Association* (AMERICAN..., 2001). The pH values of the samples were measured in triplicate using a digital potentiometer (Hanna Instruments, HI9224, Singapore, China) using the technique described by the *Instituto Adolf Lutz* (INSTITUTO..., 2008).

2.5 Statistical analysis

Two trials were carried out in the present study, one for each residue, using complete 2 × 7 factorial designs with two treatments (natural and chemically treated) for 7 storage times and three original repetitions. The results obtained were evaluated using the Statistica 7.0 software with variance analysis, regression models, and graphs, and the means for each treatment at each time were compared using the F test at 5% probability.

3 Results and discussion

3.1 pH

The pH values varied between 5.24 and 5.92 for the natural cassava bagasse, and acidified bagasse showed values between 4.7 and 5.5. At zero time, the pH of the cassava bagasse was 4.8. This possibly occurred because the pH was measured 20 minutes after processing the sample for the microbiological analyses, a period of time that was probably enough to influence the increase in the pH of the cassava bagasse.

Storage time and room temperature had a significant ($p \leq 0.05$) effect on the pH values of the cassava bagasse, with or without chemical treatment, as can be seen from the regression models, with coefficients of determination varying from 0.25 for the natural cassava bagasse and 0.74 for the acidified cassava bagasse (Table 1).

There was a linear reduction in the pH value of the natural cassava bagasse during storage at room temperature (Figure 1A), probably due to the production of acid by the fermentative microorganisms present in the sample. The lactic bacteria, exemplified by *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Leuconostoc mesenteroides* are some of the organisms most frequently found during the fermentation of cassava (LACERDA et al., 2005).

There was a significant difference between the natural and acidified cassava bagasse ($p \leq 0.05$) up to 12 h of storage, but after 24 h there was no longer any difference between them ($p > 0.05$), that is, the acid used had apparently lost its effect. This could have happened due to the buffering effect of the other acids in the samples. The buffering effect is a system of mixtures of weak acids and their conjugated bases, which confer resistance to pH changes when acid or base are added to them (CARPENTER; BROADBENT, 2009).

In the cassava peel, pH values obtained were 5.2 and 4.3 in the natural sample and 4.4 and 5.64 in the sanitized sample. Storage time and room temperature had a significant ($p \leq 0.05$) effect on the pH values of the peel samples, with or without chemical treatment, as can be seen from the regression models, with coefficients of determination varying from 0.85 for the natural peel and 0.93 for the sanitized peel (Table 1).

For the natural and sanitized cassava peel samples, the time showed a quadratic effect with a decrease in the pH value, that is, the longer the storage time, the lower the pH values, with a tendency to stabilize after 48 h at a pH of about 4.4 (Figure 1B). This decrease in the pH could be justified by the fact that the cassava peel presented a predisposition to fermentation on

Table 1. Mathematical models, level of significance (p), and determination coefficient (R²) for pH (y₁, y₂, y₃ & y₄), total coliform count (y₅, y₆, y₇ & y₈), and total yeast & mold count (y₉, y₁₀, y₁₁ & y₁₂) in the natural cassava bagasse (NCB), natural cassava peel (NCP), acidified cassava bagasse (ACB), and sanitized cassava peel (SCP), respectively, as a function of storage time at room temperature.

Treatment	Model	p	R ²
pH of NCB	$y_1 = 5.69 - 0.006 x$	0.019760	0.25
pH of ACB	$y_3 = 4.7 + 0.0106 x$	0.000001	0.74
pH of NCP	$y_2 = 5.28 - 0.04 x + 0.004 x^2$	0.000000	0.85
pH of SCP	$y_4 = 5.55 - 0.05 x + 0.0005 x^2$	0.000000	0.93
Total coliforms in NCB	$y_5 = -32915.20 + 8736.72 x$	0.000023	0.62
Total coliforms in ACB	$y_7 = 2793.96 - 583.50 x + 26.05 x^2$	0.000016	0.71
Total coliforms in NCP	$y_6 = 86172.29 - 3056.87 x + 27.42 x^2$	0.001303	0.52
Total coliforms in SCP	$y_8 = 3002.94 + 37.31 x - 1.18 x^2$	0.028684	0.33
Yeasts & molds in NCB	$y_9 = 7634.89 + 2724.02 x$	0.000001	0.72
Yeasts & molds in ACB	$y_{11} = 17943.22 + 3652.16 x$	0.000009	0.65
Yeasts & molds in NCP	$y_{10} = 26230.47 + 8233.83 x - 40.27 x^2$	0.000001	0.78
Yeasts & molds in SCP	$y_{12} = -4212.57 + 7681.83 x - 53.12 x^2$	0.000002	0.77

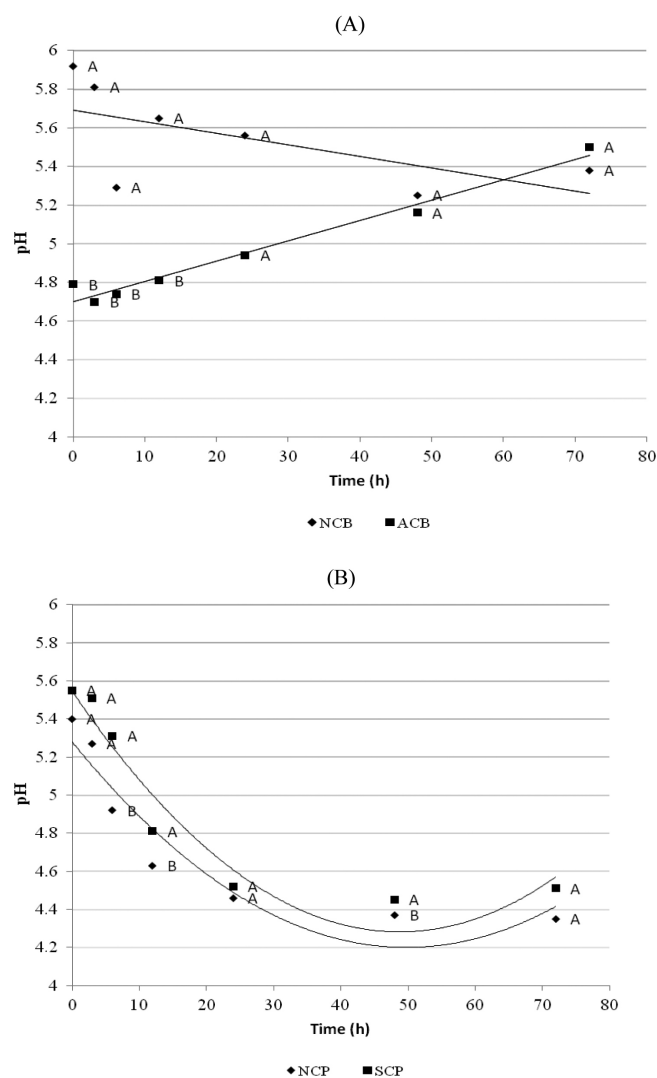


Figure 1. (A) pH of the natural (NCB) and acidified (ACB) cassava bagasse and (B) pH of the natural (NCP) and sanitized (SCP) cassava peel as a function of storage time

account of its natural characteristics. According to Araújo, Machado and Cena (2010), the pH value of the cassava root varied from 6 to 7; these values varied according to the degree of fermentation suffered by the roots as a function of time and the storage conditions, a fact also observed in the present study. There was an expected linear increase in the pH of the acidified cassava bagasse, as described earlier, due to the influence of the buffering effect of the other acids on the sample on the lactic acid, which is a weak organic acid predisposed to this type of action (BELTRAME et al., 2012).

For the cassava peel, the treatment with sodium hypochlorite presented a significant difference with respect to the pH value of the natural product at the times of 6 h, 12 h, and 48 h ($p \leq 0.05$); the sanitized cassava peel showed higher values. The pH of the natural cassava peel was already 4.9 after 6 h of storage, whereas the pH of the sanitized cassava peel reached this value only after 12 h. These results show that sanitization contributed to maintain the pH levels, that is, it inhibited fermentation of the product for a longer time, up to 12 h, since fermentation usually results in acid formation, which decreases the pH value (LACERDA et al., 2011). This is a positive effect since the cassava peels maintained a lower microbial load for a longer period of time.

3.2 Pathogenic microorganisms

Salmonella sp., *Bacillus cereus*, sulfite-reducing Clostridia, and fecal coliforms (heat resistant) were not found in any of the treatments for the different storage times at room temperature; however, total coliforms and yeasts and molds were found. The RDC n° 12 established the following standards for the microorganisms to be searched for in this type of product: *Bacillus cereus* (maximum of 5×10^3 CFU g⁻¹), Coliforms at 45 °C (maximum of 5×10^2 CFU g⁻¹), and *Salmonella* sp. (absence in 25 g) (BRASIL, 2001). The fact that these microorganisms were not found in any of the cassava peel or bagasse samples was highly positive since these organisms are pathogenic and can pose a great risk to human health in the form of infections, intoxications, and food poisoning.

Total coliforms

The total coliforms are part of a group of microorganisms that are indicators of inadequate conditions of hygiene, but which do not confer a significant risk to human health (ALVES et al., 2010). As can be seen from the regression models, the increase in the total coliform count with storage time at room temperature was significant in the case of the cassava bagasse (Table 1).

For the natural cassava bagasse, there was a linear increase in the total coliform count ($p \leq 0.05$) with storage time, varying from <10 to 6×10^5 CFU g^{-1} (Figure 2A).

However, the increase was slower for the acidified cassava bagasse. Up to 24 h of storage the count was < 10 CFU g^{-1} and after 72 h reached 1×10^5 CFU g^{-1} , that is, six times less than the count in the natural cassava bagasse (Figure 1B), again suggesting that the lactic acid was efficient in controlling the coliform population.

The delay in microbial multiplication in the lactic acid treated samples can be attributed to the effect of the lactate ions on the decontaminating solution (PIPEK et al., 2005). Organic acids have two antimicrobial effects, one is the reduction in pH to values below those of growth, and the other is the inhibition of the metabolism by non-dissociated acid molecules. It was also proven that the lactate ion can influence the energy cycle of the microorganism resulting in reduction and inhibition of bacterial multiplication (JAY, 2005).

A maximum of 24 h can be used by the factory for the processing of the acidified cassava bagasse without presenting any risks of contamination by total coliforms, as long as the process is adequately controlled.

Cereda (2002) found mean total mesophilic plate counts of 2.3×10^6 CFU g^{-1} in samples of cassava bagasse collected from factories and values of 100 CFU g^{-1} for yeasts and molds. Microorganisms capable of causing diseases, such as fecal coliforms, sulfite reducing *Clostridia* (44 °C), *Staphylococcus aureus*, and *Salmonellas* were not detected in the present study. With respect to the demands of the Brazilian legislation, according to the author, these samples could have been consumed for humans as ingredients in other food products.

For the samples of cassava peel, the total coliform count only showed significant differences ($p \leq 0.05$) between the treatments up to 12 h, suggesting that sanitization with sodium hypochlorite affected the counts in the peel and was efficient in controlling the coliform population for 12 hours (Figure 2B). The initial total coliform count in the natural cassava peel was high (8.6×10^4 CFU g^{-1}), followed by a reduction of one decimal place up to 48 h (8.1×10^3 CFU g^{-1}) and 72 h (6.8×10^3 CFU g^{-1}). For the sanitized cassava peel, there was a slight reduction in the total coliform count up to 12 h of storage at room temperature (Figure 2B). The fact that cassava is a root favors contamination by coliform since the roots are in direct contact with the soil, but according to Santos et al. (2010), if the sanitization process is efficient, the contamination should not reach the final product.

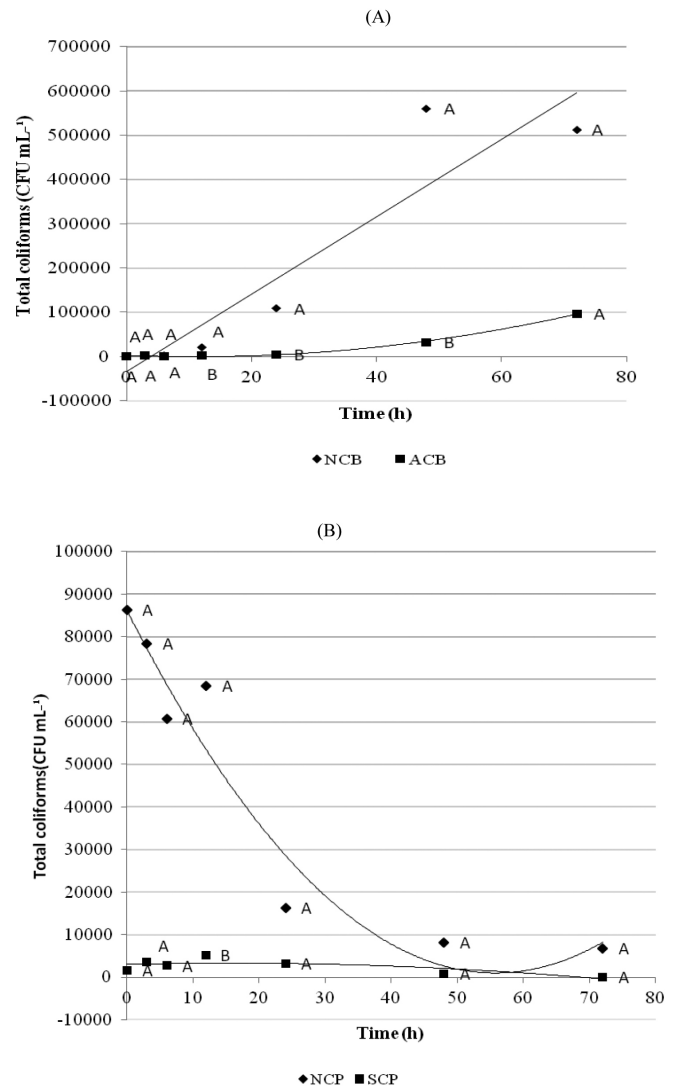


Figure 2. (A) Average counts of total coliforms in the natural (NCB) and acidified (ACB) cassava bagasse and (B) Average counts of total coliforms in the natural (NCP) and sanitized (SCP) cassava peel as a function of storage time

3.3 Yeasts and molds

Yeasts and molds, classified as deteriorative organisms, are considered a serious deteriorative threat to many products since a small fraction of these species develop well under inhospitable conditions for survival (LACERDA et al., 2011). It can be said that the acidification of the cassava bagasse was not efficient against yeasts and molds since there was no significant difference ($p > 0.05$) for the storage times evaluated (Figure 3A). Yeasts and molds show an optimum pH range for development around pH 4.0 (JAY, 2005), and thus, acidification to pH 4.5 possibly contributed to an increase in their rate of multiplication, improving the environmental conditions for their development.

In addition, no significant reduction ($p > 0.05$) was observed in the yeast and mold count of the cassava peel due to sanitization with sodium hypochlorite, despite a reduction

in the count of up to two decimal places from zero time to 12 h of storage in the chlorinated samples, followed by one decimal place after 24 h and smaller reductions for the other times. Yeast and mold counts between 2.3×10^4 and 4.2×10^5 CFU g⁻¹ were found in the natural cassava peels and between 4.4×10^2 and 2.7×10^5 CFU g⁻¹ in the sanitized cassava peels (Figure 3B).

Elevated yeast and mold counts can be explained, in part, by the fact that one of the main sources of contamination in the production system is the soil, and thus measures must be taken to reduce the microbial load (LUND et al., 2007). The regression models for the yeast and mold count in the cassava peel were also significant ($p \leq 0.05$) and presented determination coefficients of 0.78 and 0.77 for the natural and sanitized cassava peels, respectively (Table 1). The effect of storage time on the yeast and mold counts could also be visualized, showing increases for both the natural and sanitized cassava peels (Figure 3). This

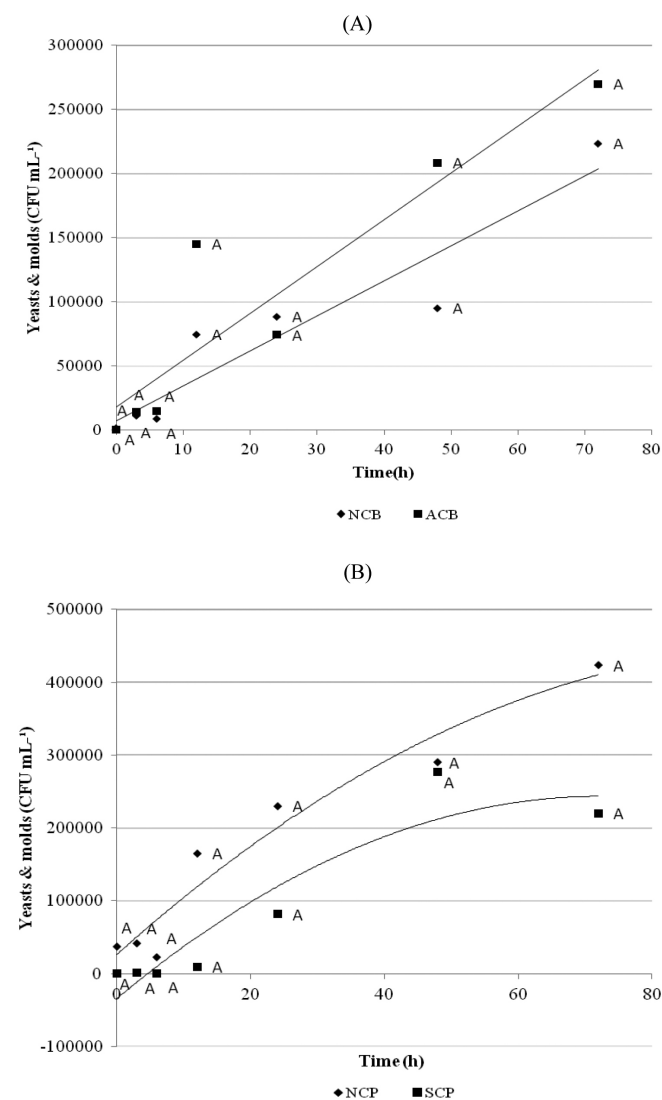


Figure 3. (A) Mean yeast & mold count in the *in natura* (BMIN) and acidified (BMA) cassava bagasse and (B) Mean yeast & mold count in the *in natura* (CMIN) and sanitized (CMS) cassava peel, as a function of storage time.

was probably favored by the reduction in the pH value of the samples, contributing positively to proliferation of the yeasts and molds, as explained previously. Many yeasts and molds are endowed with stress response mechanisms that act by reducing their susceptibility to the weak acids that accumulate at potentially toxic levels in the cells. This adaptation to weak acids, like the lactic acid, was probably an evolutionary process to facilitate growth at low pH values in the presence of organic acids (PIPER et al., 2001).

4 Conclusion

None of the samples analyzed showed the presence of pathogenic microorganisms, but the findings indicated that the conditions of hygiene were unsatisfactory in the processing of the cassava bagasse and peel, but they could easily be improved.

The acidification and sanitization applied to the cassava bagasse and peel, respectively, were effective in controlling the populations of total coliforms but not those of yeasts and molds.

The samples of cassava bagasse and peel evaluated were considered fit for human consumption as ingredients for other food products according to the microbiological standards established by the RDC n° 12 of the Brazilian Health Surveillance Agency since no pathogenic microorganisms were detected, and the yeast and mold count is not required by this legislation.

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