Stability of nut-based beverage made of Brazil nut (Bertholletia excelsa) and baru (Dipteryx alata Vogel)

Estabilidade de bebidas a base de Castanha-do-Brasil (Bertholletia excelsa) e baru (Dipteryx alata Vogel)

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ABSTRACT
People are looking for foods that promote health benefits and sometimes free of some compounds that are present because some individuals have restrictions to them, e.g. in protein foods. This trend has driven the food processing market to offer new products with a high nutritional content, low calorie, lower fat content, with specific characteristics such as lactose-free, gluten-free and convenience products, with the objective to meet the demands of consumers. In this context, water-soluble extracts appear as a plant-based beverage that have commercial and nutritional appeal and that also have been used as alternative to lactose-free assdets. Thus, the aim of this study was to process and determine the stability during storage under refrigeration of a nut-based beverage made with 9.2% (w/w) brazil nut (*Bertholletia excelsa*), 21.7% of (w/w) baru (*Dipteryx alata* Vogel), 69.1% (w/w) of water, added of 10% (w/v) of sugar, expressed in sucrose. Analysis of color, pH, titratable acidity, proximate composition, microorganisms and sensory evaluation were performed to determine the shelf life of the nut-based beverage. The beverage presented color ranging from red to yellow and complied with the microbiological standards established by current legislation, remaining microbiologically stable for at least 28 days. The water-soluble extract based on Castanha-do-Brasil and Baru obtained a high acceptance rate above 70% and achieved high scores for sensory attributes, with averages ranging between 7.09 and 7.26, on the hedonic scale, implying a shelf life greater than 28 days of storage under refrigeration.

**Keywords:** Shelf Life, Non-Dairy Milk, Water-Soluble Extract.

RESUMO
A população tem buscado alimentos que promovam benefícios a saúde, além de alguns indivíduos possuírem restrições a compostos presentes em alimentos protéicos. Essa tendência tem impulsionado o mercado de processamento de alimentos a oferecer novos produtos com alto teor nutricional, baixo teor de caloria, menor teor de gordura, com características específicas, tais como, produtos sem lactose, sem glúten e de fácil consumo, com o objetivo de atender as exigências dos consumidores. Neste contexto, surgem os extratos hidrossolúveis, sendo bebidas de origem vegetal que possuem apelo comercial e nutricional e que também têm sido utilizados como alternativa a dietas com restrição a lactose. Desta forma propôs-se com este estudo processar e determinar à estabilidade do extrato hidrossolúvel, ao longo do período de armazenamento refrigerado, composto por 69,1% (p/p) de água, 9,2% (p/p) de Castanha-do-Brasil e 21,7% (p/p) de Baru, adicionado de 10% (p/v) de açúcar, expressos em sacarose. Análises de cor, pH, acidez titulável, composição proximal, análise microbiológica e avaliação sensorial foram realizadas para determinar a vida útil do produto desenvolvido. A bebida apresentou coloração variando do vermelho ao amarelo e atendeu aos padrões microbiológicos estabelecidos pela legislação vigente, permanecendo estável microbiologicamente por pelo menos 28 dias. O extrato hidrossolúvel a base de Castanha-do-Brasil e Baru obteve um alto índice de aceitação acima de 70% e alcançou elevadas notas para os atributos sensoriais, com médias variando entre 7,09 e 7,26, na escala hedônica, implicando em uma vida útil superior a 28 dias de estocagem sob refrigeração.

**Palavras-Chave:** Vida útil; Leite vegetal; Extrato Hidrossolúvel.

1 INTRODUCTION
Foods, for being considered as complex and active systems, become susceptible to microbiological, physical and chemical changes. Its quality is a dynamic state, whose levels are continuously reducing, so it becomes necessary to study the shelf life of the product [1].
Physical analyses of foods are closely related to sensory changes, especially texture changes, which often compromise consumer acceptance [2]. Sensory analysis is an auxiliary tool of the industry, since it allows adjustments to the manufacturing of products, identifying consumer preferences, as well as in the selection of raw materials, study of shelf life, etc., optimizing and improving quality [3].

The stability of processed foods can be affected by several factors, such as raw material quality, heat treatment during processing, temperature of storage and chemical and enzymatic reactions during storage, as well as microbial changes [4]. According to Netto [5], the study of the stability of a given product can be determined by physicochemical, sensory and microbiological tests, since these are able to identify the loss of food quality. Some physical and chemical changes can also be used as parameters for the study, such as reducing the level of a nutrient or color changes [6].

Among other physical changes mentioned are the destabilization of emulsions. The structure of an emulsion results in a certain rheological behavior and colloidal interactions, which define the behavior of the emulsion under shear of the tongue and the sensation of the resulting texture [7].

Water-soluble extracts are plant based protein product defined by Brasil [8] as “foods obtained from protein parts of plant species, and may be presented in granules, powder, liquid, or other forms, with exception for those that are unconventional for food, and may be added to other ingredients as long as they do not degrade the product”. Usually these beverages require the addition of hydrocolloids which act as thickening or gelling agents like carboxymethylcellulose [3].

Maintaining the stability of emulsions and foams is one of the main problems faced by food industry. Since the destabilization of these interfaces can alter the sensory properties and consequently compromise the acceptability of the product [2]. In the elaboration of this type of beverage, it is possible to consider as a factor that helps in the conservation, the addition of the sucrose, since the sugars possess properties like humectants, agents of retention of aromas and flavors, sweetening properties and preservatives, being the latter resultant of the decrease of the free water content, due to the sucrose molecules being combined with the water molecules [9].

Due to the fact that the quality of the products is continuously reducing during the storage and the proposal of generation of a new product, it was proposed with this work, the evaluation of the acceptance rates of the nut-based beverage of brazil nut and baru according to microbiological, physicochemical and sensorial quality indicators. The evaluations were necessary to understand the behavior of the product, during the time of storage, to verify its viability of processing and biological safety.
2 MATERIAL AND METHODS

2.1 MATERIAL

Brazil nuts used in this work were, in part, donated by the company Delta Castanhas do Brasil Ltda (Iporá, GO), and another, purchased at the company Naturalista Produtos Naturais Ltda (Goiânia, GO). Brazil nut was in natura and partially unpeeled. On the other hand, the baru nuts were toasted and also from the Naturalista Produtos Naturais Ltda (Goiânia, GO).

2.2 PROCESSING OF THE NUT-BASED BEVERAGE MADE WITH BRAZIL NUT AND BARU

In previous tests, a simplex centroid design was used to determine the best proportion of water, brazil nut and baru based on the results of the sensory evaluation.

To obtain the beverage, unpeeled nuts were sanitized in chlorinated water 300 ppm for 30 minutes and rinsed with 10 ppm chlorinated water solution and then, put in an industrial blender (LSR 25, Siemsen, Brusque, Santa Catarina) with pasteurized water (80°C/25min) at 45°C in the proportion of 69.1% (w/w) of water, 9.2% (w/w) of brazil nut and 21.7% (w/w) baru, and shredded for 10 minutes to obtain a homogeneous consistent product. This product was centrifuged in a kitchen centrifuge (VCC-7000, Vicini, China), resulting in two co-products, the nut-based beverage (liquid phase) and the wet residue (solid phase) of brazil nut and baru.

After the centrifugation, the nut-based beverage obtained was submitted to a heating process at 85 °C and carboxymethylcellulose (CMC) was added along with preservatives, citric acid, potassium sorbate and sodium benzoate all in a proportion of 0.05% (w/v) and 10% (w/v) crystal sugar. Additives were necessary to give greater physical and physicochemical stability to the product, increase its biological stability and improve the sensorial characteristics, especially the flavor. It should be emphasized that the additives used in the formulation of the product met the concentrations provided in the legislation for coconut milk [10].

Subsequently, homogenization was carried out for 5 minutes and the extracts were packed in 220 ml glass bottles with crown cap and pasteurized at 80 °C for 25 minutes, and finally stored under refrigeration (4 ± 2 °C).

2.3 PROXIMATE COMPOSITION

The analyses of proximate composition of the nut-based beverage were performed on day 0 and day 28. The contents of moisture, ashes and protein (conversion factor equals 5.86) were determined according to AOAC [11] protocols, fat content according to the methodology proposed by Bligh & Dyer [12].
2.4 PHYSICAL AND PHYSICOCHEMICAL ANALYSES

The evaluation of color, pH and titratable acidity over the storage period was performed at 0, 7, 14, 21 and 28 days. Titratable acidity was determined by [13] protocols and the result expressed as percentage of oleic acid. The pH was determined by direct reading in potentiometer Micronal model B474 (Tepron Equipamentos para Laboratórios Ltda, São Paulo, SP), according to technique nº 981.12 of AOAC [11].

For determining color parameters (L *, a * and b *), a Hunterlab Colorquest II colorimeter (Hunter Associates Laboratory Inc, Reston, Virginia, United States) was used. The results were expressed in L* (luminosity) ranging from 0 (black) to 100 (white); a* varying from green (negative values) to red (positive values) and b* varying from blue (negative values) to yellow (positive values). Based on the determined coordinates, the values of chromaticity and Hue angle were calculated [14].

2.5 MICROBIOLOGICAL ANALYSES

Microbiological analyses followed the procedures described by American Public Health Association protocols [15]. The counts performed were coliforms at 35 °C, coliforms at 45 °C, molds and yeasts, coagulase positive staphylococci and Salmonella sp., established in the item 23a of Resolution RDC nº 12 for coconut milk [16].

2.6 SENSORY EVALUATION

Nut-based beverage was submitted to sensory analyses only after approval of the Research Ethics Committee (UFG) under register nº. 1.472.308. Thirty (30) milliliters portions of the beverage were randomly served in individual booths in disposable cups coded with three-digit number according to the protocol described by [13].

The overall acceptance of the product was estimated using a 9-point hedonic scale in which the taster assigned scores of 1 (I detested) to 9 (I loved), for the attributes color, odor, taste and overall impression. For purchase intention test a scale ranging from 1 (Certainly would not buy) to 5 (Certainly I would buy) was used.

With the data obtained in the tests of acceptability, acceptability indexes were calculated for all treatments using Equation 1:

\[
AI = \frac{A \times 100}{B}
\]  

(1)

where A is the mean score obtained for the product and B is the maximum score to evaluate the product. Values equal to or higher than 70% can be considered good acceptance according to Dutcosky's methodology [17].
2.7 STATISTICAL ANALYSES

The experimental data of the physical and physicochemical analyses and sensory evaluation were submitted to analysis of Variance (ANOVA) and comparison of means by the Tukey's test, at a level of 5% significance (p≤0.05), with the aid of Statistica software. The results of the Proximate Composition were submitted to t-test was used to verify if there was a significant difference between the means at the 5% level (p≤0.05) using Statistica software.

3 RESULTS AND DISCUSSION

3.1 PROXIMATE COMPOSITION

Proximate composition of the best formulation of the nut-based beverage made with brazil nut and baru are presented in Table 1.

Table 1: Proximate composition of nut-based beverage made with brazil nut (Bertholletia excelsa) and baru (Dipteryx alata Vogel).

| Time (Days) | Moisture* | Protein* | Fat*     | Ash*    | Carbohydrates* |
|------------|-----------|----------|----------|---------|----------------|
| 0          | 64.86± 0.02 | 6.34± 0.15 | 13.06± 0.06 | 0.84± 0.01 | 14.90± 0.23 |
| 28         | 64.86± 0.01 | 5.67b± 0.06 | 12.75b± 0.07 | 0.77b± 0.02 | 15.94a± 0.10 |

Mean of three determinations. Carbohydrates = [100 - (protein + moisture + ash + fat)]. *expressed in g/100g. Means followed by different letters in the same column differ significantly from each other at a level of 5% p (≤0.05) by the t-test.

It is observed that moisture contents remained constant, not presenting a significant difference at the level of 5% (p<0.05) and the contents of protein, fat and ashes decreased throughout the storage. Consequently, it reflected in an increase in carbohydrates content since these were calculated by difference.

The reduction of fat content can be justified due to auto-oxidation and photo-oxidation reactions, favored by being a product with a high amount of lipids [18]. However, it is necessary to study the enzymatic profile of brazil nut and baru to better understand these changes in the proximate composition.

D'Oliveira [19] developed a beverage flavored with baru and Morais [20] elaborated a water-soluble extract made with cashew nut. These authors obtained lower amounts of proteins than those reported by the present study, being 4.74% and 3.87%, respectively. The fat content obtained in this study was higher when compared with the data reported by the same authors, of 4.26% and 6.20%, in that order. However, the fat content found in this study was much lower than that obtained by Santos [21] for brazil nut water-soluble extract, which found 74%. Such variations occur due to the
different nuts used, as well as the many proportions involved in the process of obtaining the water-soluble extracts.

Based on the data obtained, the nut-based beverage made with brazil nut and baru are in consonance with the parameters described in the current legislation, which establishes the identity and minimum quality characteristics that must be met by plant-based protein products (RDC No. 268, September 22, 2005), which recommends the minimum amount of protein as being 3.00% (g.100 mL−1) [8].

The most produced and consumed vegetable water-soluble extract is soybean water-soluble extract [19]. Comparing to soybean water-soluble extract obtained by Maia et al., [22], it was observed that the authors produced a beverage with high moisture content (93.60%) and lower contents of protein, as well as lipids, ashes and carbohydrates (3.20%, 3.00%, 0.30% and 1.30%), respectively.

Therefore, it is concluded that the nut-based beverage made with brazil nut and baru presented a higher protein potential than the soybean water-soluble extracts produced in the studies of the mentioned authors.

The pH values increased significantly, at a level of 5% (p <0.05), from the first to the second week, then falling on the third week and remaining practically stable up to 28 days. The titratable total acidity showed a correlation with the pH behavior, with a marked decrease from the first to the second week, and then increased in the third week, remaining stable until the end of the storage, being inversely proportional to pH, as can be verified in Figure 1.

![Figure 1: Evolution of the physicochemical parameters of the nut-based beverage made with brazil nut (Bertholletia excelsa) and baru (Dipteryx alata Vogel) during storage.](image-url)
This behavior of variation in pH and acidity was also observed by Carneiro and Arévalo-Pinedo [23] in a study of the shelf life of beverage made with babaçu (*Orbignya phalerata*) water-soluble extract and brazil nut water-soluble extract added of 11% sugar. Cardarelli and Oliveira [24] reported similar behavior in a study of the shelf life of Brazil nut water-soluble extract, observing the decrease of pH for all formulations studied.

Santos [21], studying the stability of brazil nut water-soluble extract, observed that treatments containing preservatives kept the pH practically stable throughout the storage, presenting only a small decrease at the end of the storage, with similar mean values of this study (5.96). Morais [20] found the mean pH for the cashew nut almond water-soluble extract of 6.09, quite close to the values observed in this experiment.

### 3.2 PHYSICAL AND PHYSICOCHEMICAL STABILITY

In Table 2 is presented the behavior of the variations in luminosity, chromaticity and Hue angle of the nut-based beverage during the 28 days of storage.

| Day | Luminosity       | Chromaticity | Hue angle*   |
|-----|------------------|--------------|--------------|
| 0   | 65.69\textsuperscript{c} ± 0.12 | 19.31\textsuperscript{d} ± 0.02 | 75.37\textsuperscript{a} ± 0.04 |
| 7   | 64.97\textsuperscript{d} ± 0.09 | 19.15\textsuperscript{c} ± 0.09 | 75.41\textsuperscript{a} ± 0.10 |
| 14  | 68.59\textsuperscript{a} ± 0.08 | 20.92\textsuperscript{b} ± 0.04 | 75.02\textsuperscript{b} ± 0.02 |
| 21  | 68.47\textsuperscript{a} ± 0.04 | 21.24\textsuperscript{b} ± 0.04 | 75.00\textsuperscript{b} ± 0.04 |
| 28  | 67.40\textsuperscript{b} ± 0.03 | 20.75\textsuperscript{b} ± 0.05 | 75.23\textsuperscript{a} ± 0.04 |
| Means | 67.02 ± 1.63 | 20.27 ± 0.97 | 75.23 ± 0.21 |

Means followed by different letters in the same column differ significantly from each other at a level of 5% p (≤0.05) by Tukey’s test. *in degrees.

In Table 2 are shown the values of luminosity ranging from 64.97 to 68.59, demonstrating that the nut-based beverage made with brazil nut and baru are more distant from white (100).

Carneiro and Arévalo-Pinedo [23], found a value of 73.35 for the luminosity attribute of the beverage made with 60% of babaçu (*Orbignya phalerata*) and 40% of brazil nut. These variations are mainly due to the characteristics of the different raw materials used for the production of beverages.

It was observed that chromaticity of the product increased significantly (p <0.05), ranging from 19.15 to 20.75, showing that the product presented a more intense color, that is, distancing more from the gray color.
The Hue angle, which indicates color saturation, remained stable during storage, did not show a significant difference (p > 0.05) between the first (7th day) and the last storage day (28th day). The results obtained for this parameter correspond to values close to 90°, resulting in a beverage ranging from red to yellow.

Therefore, the nut-based beverage made with brazil nut and baru analyzed in this work presented a yellowish-white coloring tending to red according to the values found for luminosity, chromaticity and hue angle (Table 2).

3.3 MICROBIOLOGICAL ANALYSES

The microbiological analyses of the samples preceded the sensory evaluation tests and were performed according to the recommendations of the National Health Surveillance Agency (ANVISA), RDC No. 12 [16].

Considering that the nut-based beverage made with brazil nut and baru and the "coconut milk" are similar products, these analyses were based on the standards of the item 23.a) which requires the tests of coliforms at 45°C, coagulase positive staphylococci and Salmonella sp. Molds and yeasts analysis is not required by legislation, but considering the need for constant improvement of sanitary control actions in the food area and aiming, this counting was also carried out. In Table 3 are shown the results obtained in the microbiological analyses of the nut-based beverage during the storage.

Table 3: Count of microorganisms of nut-based beverage made with brazil nut and baru during 28 days of storage.

| Time (Days) | Coliforms at 35°C* | Coliforms at 45°C* | Molds and yeasts* | Salmonella sp.** | Coagulase Positive Staphylococci* |
|-------------|-------------------|-------------------|-------------------|------------------|----------------------------------|
| 0           | < 10              | < 10              | <10               | Absent           | < 10                             |
| 7           | < 10              | < 10              | < 10              | Absent           | < 10                             |
| 14          | < 10              | < 10              | < 10              | Absent           | < 10                             |
| 21          | < 10              | < 10              | < 10              | Absent           | < 10                             |
| 28          | < 10              | < 10              | < 10              | Absent           | < 10                             |

* Colony-forming unit per mL of sample (CFU/mL). ** Absence in 25 mL of sample. Maximum values permitted (RDC nº12/2001): Coliforms at 45°C: 10² CFU/mL; Staphylococci: 10³ CFU/mL; Salmonella sp.: absence in 25 ml

The evaluated samples were in agreement with the microbiological standards required by the current legislation [16], for coconut milk, demonstrating good hygienic-sanitary conditions and adequate heat treatments to guarantee food safety. It is concluded that the nut-based beverage made with brazil nut and baru remains microbiologically stable for at least 28 days.
3.4 SENSORY EVALUATION

The criterion used to determine the shelf life of the nut-based beverage made with brazil nut and baru was established crossing the responses obtained from the microbiological analyses and the sensorial parameters, with the intention that the time determined as the end of the shelf life did not cause harm to consumer health.

The shelf life was determined based on the score of the sensory evaluation, where the closer to zero is the score attributed to the product, the more inappropriate it is to be consumed. The score of 6.0 or less (slightly like) on the overall impression scale of the sensory analysis was the cut-off point that defined the end of the shelf life of the nut-based beverage made with brazil nut and baru [25]. The results of the acceptance and purchase intention tests performed by the panelists during the 28 days of refrigerated storage can be seen in Table 4.

Table 4: Sensory evaluation of nut-based beverage made with brazil nut (*Bertholletia excelsa*) and baru (*Dipteryx alata* Vog.) during 28 days of storage.

| Day | Odor    | Color   | Taste   | Overall impression | Purchase intention |
|-----|---------|---------|---------|--------------------|--------------------|
| 0   | 7.53^a  ± 7.30^a | ± 8.20^a ± 7.93^a | ± 4.16±  |                   |
| 7   | 7.47^a  ± 7.33^a | ± 6.70^b ± 6.90^ab | ± 3.83^a ±  |                   |
| 14  | 7.47^a  ± 6.63^a | ± 7.23^ab ± 7.27^ab | ± 3.70^a ±  |                   |
| 21  | 6.77^a  ± 7.20^a | ± 7.40^ab ± 7.30^ab | ± 3.97^a ±  |                   |
| 28  | 7.00^a  ± 7.00^a | ± 6.77^b ± 6.77^b | ± 3.60^a ±  |                   |

Average: 7.25 ± 0.34 7.09 ± 0.29 7.26 ± 0.60 7.23 ± 0.45 3.85 ± 0.22

Means followed by different letters in the same column differ significantly from each other at a level of 5% p (≤0.05) by Tukey’s test.

The nut-based beverage made with brazil nut and baru presented good acceptability, with approximate averages varying from 7.09 to 7.26 for acceptance attributes (odor, color, flavor and overall impression), placing them among the categories “moderately like” and “like very much”. It was observed that the beverage obtained averages always above 6 throughout all the storage, consequently it is concluded that the shelf life, based on the sensorial and microbiological evaluations, does not end on the 28th day of storage under refrigeration, according to the orientation of Paiva et al. [25].

Santos [21] studying the stability of Brazil nut water-soluble extract observed that extracts with preservatives had 12 days of shelf life which is shorter than the results obtained in this study, 28 days.
Odor and color attributes showed no significant difference over 28 days, with means ranging from 6.77 to 7.53 and 6.63 and 7.33, respectively, situating between “slightly like” and "moderately like". Flavor attribute presented a significant decrease from day zero to the day seven, however remained stable over the last 21 days of storage and showed no significant difference (p <0.05). The average of this parameter varied between 6.77 and 8.2, situating between "slightly like" and "like very much".

Santos [21] analyzing brazil nut water-soluble extract, using a hedonic scale of 9 points, presented averages for the global acceptance attribute between the categories “liked slightly” and “moderately like”, corresponding to the score between 6 and 7 on the hedonic scale. Thus, the nut-based beverage made with brazil nut and baru developed in this study, obtained superior acceptability in relation to the brazil nut water-soluble extract. Ferberg et al. [26] verified that the nut extract with 3% of sugar reached a higher frequency of notes 6, 7 and 8 among the treatments studied, values similar to those obtained in this study.

Purchase intention, a parameter evaluated in this study using a scale ranging from 1 (Certainly would not buy) to 5 (Certainly would buy), obtained a high consumer acceptance index, with means ranging from the hedonic terms "Maybe bought/maybe did not buy" to "Certainly Buy" and did not present significant differences between them (p <0.05). For each attribute analyzed, the acceptability index (IA) was calculated and the results are shown in Figure 2.

![Acceptability index of nut-based beverage made with brazil nut (Bertholletia excelsa) and baru (Dipteryx alata Vog.) during storage.](image)
All the attributes analyzed for the nut-based beverage made with brazil nut and baru obtained values of acceptability index (AI) higher than 70%. According to Dutscosky [17], acceptability indexes higher than 70% are considered good indexes for commercialization, therefore the nut-based beverage was well accepted by the judges. Studies carried out to evaluate the development of chocolate drinks, made from water soluble extracts of soybean and cheese whey, for color, taste and consistency attributes, obtaining mean values of acceptability indexes ranging from 45% and 82%, indices and averages lower than that found in the present study [27].

Oliveira et al. [28] carried out studies on soybean extract and fruit juices with different concentrations and presented a global acceptance means of 4.8 for beverage made of soybean (Glycine max) and açaí (Euterpe oleracea) extracts. Once these means are lower than those obtained in the present study, nut-based beverage made with brazil nut and baru had greater acceptability.

4 CONCLUSIONS

In terms of composition, the nut-based beverage made with brazil nut and baru had a better performance related to nutritional value compared to the soybean extract. Due to the excellent nutritional characteristics presented, with the advantage of being lactose-free, this nut-based beverage can be a good alternative protein intake for people with lactose intolerance.

The use of pasteurization and refrigeration allowed the product to remain stable microbiologically for at least 28 days. The absence of total coliform and thermotolerant groups ensured the microbiological quality of the nut-based beverage, showing that the hygiene conditions in processing and storage of the product were considered satisfactory.

The nut-based beverage obtained had high acceptance rate, above 70%, and achieved high grades for sensory attributes, therefore it is concluded that the shelf life, based on the sensory evaluation tests and microbiological analyses ratings, does not end on the 28th day of storage under refrigeration.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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