New data on amylolytic fermentation in the Amazon

Novos dados sobre a fermentação amilolítica na Amazônia

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Abstract: In a previous article (Barghini, 2020), it was shown that the amylolytic fermentation of carbohydrates to produce alcoholic beverages was widespread from Guianas to the upper Amazon, in Venezuela and Colombia, and reached the Misquitos, Central America. However, of the 43 reports studied, only one referred to the right side of the Amazon River, and only a single biochemical analysis of the resulting products was found. In this article, the analysis of two products: *tiquira* and *tarubá*, widespread in the local culture and the national culture, but of indigenous origin, allowed us to expand our knowledge about the geographic reach of this process. The significant number of biochemical analyses available for these two products also broadened our understanding of the Amazon basin's fermentation processes. It partly extended the results to other processes described by missionaries and chroniclers, in general, neglected by anthropologists. Based on the new data, it is possible to affirm that fermentation processes had a fundamental role in the conservation and transformation of food in environments such as tropical forests, where the climate favors especially aggressive food degradation by bacteria, molds, and insects.

Keywords: Fermented beverages. Amylolytic fermentation. *Aspergilus* sp. *Neurospora* sp. *Rhizopus* sp.

Resumo: Num artigo anterior (Barghini, 2020), foi mostrado que a fermentação amilolítica dos carboidratos para a produção de bebidas alcoólicas era difundida das Guianas até o alto Amazonas, cobrindo também Venezuela e Colômbia e atingindo, com os Misquitos, a América Central. Dos 43 documentos consultados, apenas um se referia à margem direita do rio Amazonas, e foi possível encontrar uma única análise bioquímica dos produtos resultantes. A análise de dois produtos, a tiquira e o tarubá, difundidos na cultura local e na cultura nacional, mas de origem indígena, permitiu ampliar o alcance do uso deste processo. A disponibilidade de um número significativo de análises bioquímicas dos dois produtos permitiu ampliar a compreensão dos processos fermentativos na bacia amazônica, e estender, em parte, os resultados a outros processos descritos por missionários e cronistas, mas aos quais foi dada pouca atenção por parte dos pesquisadores. Com base nos novos dados, é possível afirmar que os processos fermentativos tiveram um papel fundamental na conservação e transformação de alimentos em um clima especialmente agressivo, como o encontrado nas florestas tropicais, que tende a degradar os alimentos por meio da ação de bactérias, fungos e insetos.

Palavras-chave: Bebidas fermentadas. Fermentação amilolítica. *Aspergilus* sp. *Neurospora* sp. *Rhizopus* sp.

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INTRODUCTION

In a previous article, Barghini (2020) studied the diffusion of the process of amylolytic fermentation of complex carbohydrates to produce fermented beverages. As highlighted on that occasion, complex carbohydrates are relatively stable, making fermentation and bacterial attack harder. Thus, before alcoholic or lactic fermentation occurs, it is first necessary to perform saccharification of the starches. Traditionally, it was believed that the production of beverages from amylaceous substances in the South American lowlands took place only through the introduction of salivary amylase in the fermentation mass – in practice, through chewing. In 2004, Henkel (2004, 2005) performed a biochemical analysis of *pajuaru*, a beverage made by the Wapisiana indigenous group, and verified that the saccharification of starches was achieved using a mold (*Rhizopus* sp.) with amylolytic properties. Henkel sustained that this was the only case of this type of process in South America. Amylolytic fermentation is a process of saccharification of carbohydrates widely used in China, Japan, the Philippines, and South East Asia to produce alcoholic beverages, sauces, and food, but had not been registered in other regions of the world. However, in the previous article (Barghini, 2020), by consulting reports from missionaries, travelers, and anthropologists, it was possible to identify 43 occurrences of this type of saccharification across a large area from Guyana to the Upper Amazon, Venezuela, Colombia, and reaching Central America. The process was practiced by different linguistic groups, i.e., Arawak, Carib, Chibcha, Kichwa, Miskito, Ticuna, Tukano, Tupi, and others unidentified. The name of the fermented beverage differed according to the linguistic group, but in the Guianas and the Rio Negro, the terms *parakari* and *pajuaru* dominated. Curiously, only one of the cases compiled in Barghini (2020) referred to an indigenous group located on the right side of the Amazon River – in all of the other reports, when it was possible to locate the case, the location was clearly on the left side of the Amazon. While some reports (e.g., Peckolt, 1878) stated that the process was widespread throughout the Amazon, they often did not indicate where it actually took place. This fact appeared anomalous because the Amazon River does not represent a barrier to cultural diffusion, and there are no substantial differences between the two sides of the river from a biogeographical point of view.

In Barghini (2020), only reports regarding processes conducted by traditional indigenous groups were used. Since then, while consulting the folkloristic literature of the region of Santarém and Maranhão, it was possible to find two traditional products, *tiquira* and *tarubá*, diffused mainly among the traditional population of these regions, which also involve the saccharification of starches with amylolytic molds. Furthermore, published biochemical tests of both these products are available in the literature. Since only one biochemical analysis was found among the cases studied in Barghini (2020), this paper presents an in-depth study of *tiquira* and *tarubá* with the goal of enriching the understanding of fermentative processes in the Amazon.

Tiquira is distilled from an alcoholic fermented manioc wine, still in use mainly in the states of Maranhão, Alagoas, and Piauí. *Tarubá* is a drink that can be nonalcoholic or slightly alcoholic. *Tarubá* is popular in Pará but is also used in many Amazon regions. Both products are of clear indigenous origin, although the caboclo population uses them today.

One last point that should be highlighted is that these are processes that developed in equatorial environments which, according to the classification in the Köppen-Geiger system, vary between Af, a totally humid tropical climate, and Am, the monsoons, with two months of drought, and small inclusions of the Aw climate, or dry winter. Throughout the year, the average temperature variation is less than the daily fluctuation. This means that temperature and humidity are relatively stable so that the processes can be conducted year-round without a marked seasonality. To ensure their continuity, temperature

stability is fundamental in all fermentation processes, so industrial fermentations are performed in environments with thermostatic control. This consideration is important when considering why the process probably did not spread to the coast in pre-colonial times. According to the Köppen-Geiger system, Espirito Santo's coastal state varies between Cfa Humid subtropical and Cfb Oceanic climates. On the other hand, we cannot completely rule out the existence of this molding technique on the Brazilian coast based only on the fact that the chroniclers did not report it.

METHODOLOGY

The purpose of this paper is to use contemporary scientific data to analyze and interpret old processes for which we do not have a scientific survey or archaeological material to test the interpretation. Since we cannot be sure of these two traditional products' antiquity, we will first analyze the written sources to prove their authenticity. Secondly, data from the analysis will be used to describe the process from a scientific point of view. When concluding, we explore the meaning of the biological results.

This research was essentially a bibliographic search to identify the names *tiquira* and *tarubá* in anthropological literature and then check modern mentions of these two products, focusing on biological analysis[1].

This article is structured as follows: each preparation is addressed, starting from different testimonials to identify its indigenous origin. A review of the main studies conducted recently on the biological characterization of the processes is then presented. As these are processes of industrial interest, specific studies are still being conducted in an environment technically and culturally close to the native environment. After describing these analyses, the implications of the results for the interpretation of the fermentations in the rest of the Amazon are discussed.

Map reproduces the locations of the processes identified in Barghini (2020) alongside those described in the current study (Figure 1).

TIQUIRA: HISTORY

Tiquira is a distillate of a cassava wine obtained with the saccharification of starches with amylolytic molds. It is a Brazilian regional product with a strong local connotation, evoked in the words of novelists such as Jorge Amado and poets such as José Sarney, and it is often attributed to an indigenous origin (Lima, 1943, p. 1975; Cereda & Costa, 2008; Cereda & Brito, 2018).

In Maranhão, the traditional process of *tiquira* production was conducted until a few years ago by many small producers in facilities that were not very different from those that could be found in an indigenous village. Cereda and Costa (2008, pp. 20-29) have described the traditional production process in detail and documented the extremely simple equipment used. The first phase, the production of the wine to be distilled, was similar to the production of *pajuaru*, one of the fermented beverages mentioned in Barghini (2020). Cassava was grated as finely as possible. The dough was passed through a press or a *tipiti* to remove the *manipueira* (the liquid extracted from the mass). To prepare *beijus* (cassava bread), the mass was dried on a heated plate at about 70 °C to form cakes 30-35 cm in diameter and 8-10 cm thick (in the indigenous tradition, *beiju* for the production of *pajuaru* is called *beijuguaçu*, or big *beiju*). The *beiju* should be 'toasted on both sides' and have a golden color. For mold formation, they needed to have humidity that, once cooled, was between 30% and 35%. After a rest period of 18-24 h, the *beijus* would remain for 8-9 days in a place with minimum light and ventilation, warmed on jiraus (wood platforms), and covered with palm or banana leaves. In an alternative process, after toasting, the *beijus* would be placed on the ground on a bed of banana or palm leaves and again covered with leaves.

[1] The research resulted in the discovery of six more instances of amylolytic fermentation: four in Ecuador, in the region of the montaña, among Yorimagua, the Canelos Kichwa, and the Quichua speakers of the Curaray River, one in Costa Rica, among the Bribri, and one among the Ticuna, on the right side of the Amazon River, near the boundary of Brazil and Colombia.

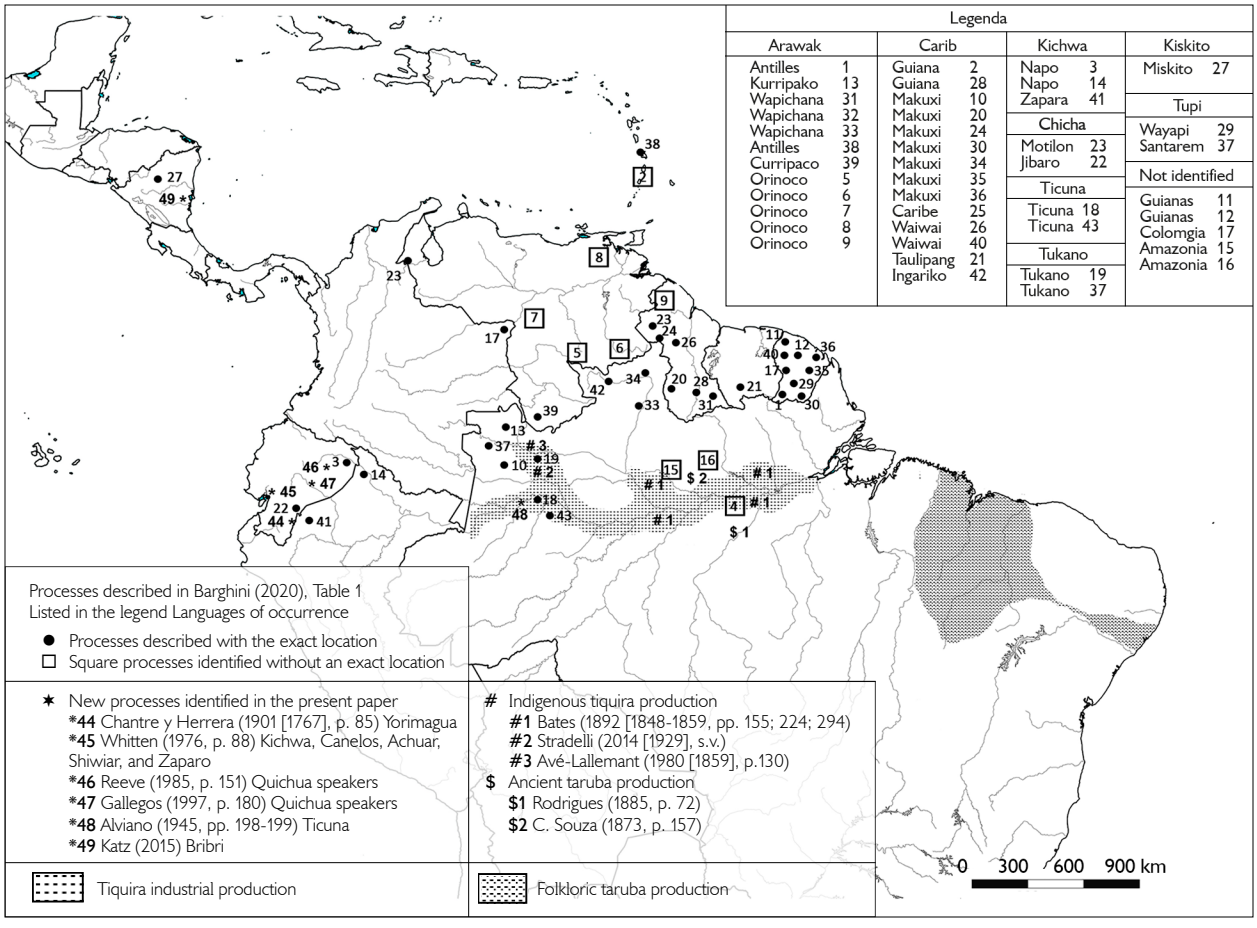

Figure 1. Map of the distribution of amylolytic saccharification processes in the Amazon and Central America. Map: modified by Barghini (2020).

The moldy *beijus* could be used immediately for fermentation or be left to dry for later use. For alcoholic fermentation, the *beijus* were crumbled in water, 1 kg of *beiju* per 4 l of water, and left to ferment for 7 days. After fermentation, the 'wine' was filtered and distilled in a distiller made of clay or copper (Cereda & Costa, 2008, pp. 20-29).

Based on descriptions of the process and photographs of the equipment reproduced in Cereda and Costa (2008), it is possible to understand that *tiquira* could be produced in indigenous communities without access to modern technology.

Henry Koster' detailed description of a still house in 18th century Pernambuco explains how simple and easy to reproduce the equipment could be:

> The stills are earthen jars with small necks, and likewise small at the bottom, widening upwards considerably, but again straightening on approaching the neck. The foundation of a circular oven is formed, and two of these jars are placed within it, one on each side of it, in a slanting position, with the bottom within the oven and the neck on the outside, and being thus secured the walls of the oven are built up against them, and the top is closed in. These stills have round caps, carapuças, which fit on to the mouths of the jars, and are rendered perfectly tight by a coat of clay being daubed round the edges, alter the wash has been put into the still and the wash has been lighted underneath. These caps have on one side a pipe of six inches in length attached to each of them, and into this is inserted the end of a brass tube of four feet in length. This tube is placed in a broad and deep earthen pot or jar containing cold water, and the opposite end of it reaches beyond the pot. The tube is fixed with a sufficient slant to allow of the liquor running freely through it (Koster, 1917 [1810?], pp. 150-151).

In fact, there is a record of indigenous communities that practiced distillation, but probably at an early stage; it was a union of indigenous technology to produce 'wine' with Portuguese distillation technology and was initially practiced by Portuguese settlers. A review of the sources will be useful to clarify this. Some authors state that Piso and Marggraf (1648) have already described distillation among the natives and that the name *tiquira* was used in '*Historiae rerum naturalium Brasiliae*'. Despite citing cassava 'wines', Piso and Marggraf (1648, pp. 54-274) do not explicitly quote *tiquira*, and only refer to two ways to ferment cassava, either by chewing or 'beating'. They do not mention *tiquira* or the distillation process. They probably misunderstood the term '*cagassa*' (*cachaça*) used by Piso and Marggraf (1648, p. 51) as referring to the current sugar-cane-based spirit of the same name. In the 17th century, the term 'cachaça' referred to the foam released from the purification of sugar cane juice, which was given to animals (see also Antonil, 1837 [1711], p. 77).

Distilled beverages were common in colonial Brazil. The spirits were initially imported and called '*aguardente do reino*' (firewater [spirit] from the kingdom [Portugal]); when of local origin, they were called '*aguardente da terra*' (firewater [spirit] from the land) and were produced mainly with sugarcane, but also with manioc. In the latter case, they were called '*aguardente de beiju*' (firewater [spirit] from cassava bread). However, in 1653, the production of cassava wine distillate was forbidden, and the distilled spirit from sugarcane was taxed at R$ 400 for each '*canada*' (Varnhagen, 1877?, p. 729) (ancient Portuguese unit of volume equivalent to 1.4 liters).

However, the natives did not produce the spirits. Father Daniel (2004 [1750], p. 526) commented on the '*contratto da aguardente*' (contract of spirits), by which the settlers, in exchange for the spirits, ". . . have as much of them as they want; and if the whites set aside their consciences, with a bottle they fill a boat with other drugs". That the natives did not produce spirits is reinforced by the fact that the '*Diretório dos índios*' (Mendonça Furtado, 1758), in articles 40, 41, and 42, strictly regulate the commerce and transport of spirits, to prevent it from being supplied to the natives (Cf. Tanan, 2019).

Alexandre Rodrigues Ferreira repeatedly comments, in his diaries, on the problem of the spirits and the prohibition on importing the kingdom's spirits, stating that the settlers cultivated cassava to produce spirits and not food (Ferreira, 1888, p. 65) and that the city halls charged the producers a tax.

The first mention of a distilled drink with the name *tykyra*, or *tiquira*, is found in the Tupi dictionary of 'Beitrãge zur Ethnographie und Sprachenkunde Amerika's zumal Brasiliens', by Martius (1867a [1817-1820], p. 92, 1867b [1817-1820]). It contains the definition, in Portuguese and German, '*tykyra - agoa ardente de farinha de pãoo, Branntwejn aus Mandiocca-Mehl*' (spirit from cassava flour). However, when one consults the ethnography, Martius (1867a [1817-1820], p. 712, 1867b [1817-1820]) recognizes that the natives knew the distilled beverages, giving a name '*tykyra*,' from the '*tykyr*' (drip), but did not have the technology to make it. To compensate, they tried to make the fermented beverages more alcoholic. Interestingly, in Brazilian Portuguese, the common term for spirit, besides '*cachaça*' and '*aguardente*,' is '*pinga'* (drop), a metaphor analogous to that used in Tupi.

In his travels as a naturalist on the Amazon River, Henry Walter Bates mentions distilled beverages on three separate occasions. The first time was in Óbidos (Bates, 1892 [1848-1859], pp. 155-156). The second time was on the Tapajos river, and he only mentions liquor, defined as '*aguardente de beiju*' [cassava spirit] (Bates, 1892 [1848-1859], p. 224). The third time was in Tefé, for which Bates uses the old name *Ega*. Here, Bates (1892 [1848-1859], p. 280) met an indigenous group, which performed distillation with clay distillers, but he writes: "They learned the art of distilling spirits from the early Portuguese; it is only, one or two of the superior tribes, such as the Juris and the Passés, who practice it" (Bates, 1892 [1848-1859], p. 156). The city of Tefé, although located well in the interior of

the Amazon, on the right side of the Solimões, 600 km upstream from Manaus, was a missionary center of the Portuguese Carmelites as early as 1620, and the city of Ega, currently Tefé, was one of the villages founded by the missionaries around 1751. The populations, mainly indigenous, who lived in the city, had therefore suffered a deep Portuguese influence.

The account of artillery captain Joaquim Firmino Xavier, reproduced by Avé-Lallemant (1980 [1859], pp. 124-137), describes in detail the indigenous villages' state of neglect in the upper Negro River because of messianic movements. The governor of Manaus founded the villages with the purpose of 'reducing' the natives to 'civilization'. In the context of this state of neglect, we find mention in the village of Sta. Ana (Avé-Lallemant (1980 [1859], p. 130), of drinks distilled from manioc and sugarcane.

Until the mid-1800s, therefore, the art of distillation was extremely limited among the natives. In the rest of Brazil, mainly in Maranhão, in the middle of the 18th century, the production of cassava spirit, called *tiquira*, was already an industrial activity. In the catalog of the Universal Exhibition of Rio de Janeiro of 1861 (Catalogo, 1862), there is a very detailed description of the process, considered an industrialized product of the country.

A few years later, in his book "Apontamentos para o dicionário histórico geográfico e estatístico da província do Maranhão", Cezar Augusto Marques describes the process, and offers statistics for the production of *tiquira* per *comarca* (district). He also comments on how industrial production survived, despite the prohibition of the production of brandy contained in the *carta regia* (royal decree) of February 24, 1647 (Marques, 1864, p. 171). Therefore, the 'industrial' production of manioc brandy was commercialized when Brazil was a Colony and after it became an Empire.

In 1894, Rodrigues (1894, s. v.) provided the following definition of *Tykyra*: "from ty, broth, and kir, green, fresh. It is a brandy made with fresh cassava stock". However, the *Tykyre* entry is "verb, drip, drip".

The existence of distilleries for native spirits has been confirmed based on, in addition to the reports by Bates and Joaquim Firmino Xavier mentioned above, Stradelli (2014 [1929], s.v.) dictionary. In the entry '*alembic*', *mutykyrepaua* is described as an alembic, made entirely of clay and wood, and using an alcohol condensation system made with pieces of spliced bamboo. In his description of the system, Stradelli comments that it was 'primitive' and notes how the fact that the natives did not use sugarcane indicates a native origin of the process. The dictionary also registers different uses of the term *tykyra*. Stradelli spent the last years of his life (from 1912 to 1920, before he suffered from leprosy) completing his dictionary as a judge of peace in Tefé, exactly where Bates had observed Indians who distilled with clay distillers. It is possible that during the rubber boom, when a deep disruption of indigenous society took place even in the most isolated regions of the Amazon, some groups started to conduct distillation inspired by the settlers' experiences. If true, this effect would have been recent because Spruce (1908 [1849-1864]) and Wallace (1853), who traveled through the area and left reports of the habits of the natives, only commented on the great attraction that distilled beverages exerted for them, but never spoke of indigenous distillation. Lowie (1948, p. 46) and Steward (1948, p. 529) confirmed that distillation was a recent phenomenon and commented that bamboo was used among the Quijos in the cooling circuits of the stills.

It is essential to point out that the word *tykyra* means "what falls, drop by drop", Alviano's (1945, pp. 198-199) description of *pajuaru* production is quite explicit and could imply that in some cases, it was produced in drops. In the process described by Father Alviano (not listed Barghini, 2020), the *beijus*, after being left to mold for 3 days covered with leaves, are placed in an *igaçaba* (clay container, typically with a wide mouth) partially suspended on pieces of wood and drops of cold water are dropped on them. Within two days, *pajuaru* collects at the bottom of the container. In this process, saccharification of the starches with amylolytic molds and alcoholic fermentation with natural yeasts occur

in parallel. In industrial fermentations, in general, the two processes take place in two stages, saccharification then alcoholic fermentation; only in Japan the production of *saké* is performed in parallel.

BIOLOGICAL ANALYSES OF THE PRODUCTION PROCESSES OF *TIQUIRA*

Currently, *tiquira* is an industrialized product prepared with modern processes, partly due to the effort of *Empresa Brasileira de Pesquisa Agropecuária* (EMBRAPA) and other scientists to develop more rigorous criteria in production, using enzymes and industrial processes (Cereda & Costa, 2008). In an effort to modernize production, numerous studies supply information on the traditional fermentation process.

Most research was intended to find mechanisms to optimize the process using commercial enzymes. Hence, such studies elicit limited interest from an anthropological point of view. Others, however, despite being oriented toward optimizing processes, ensured wide documentation in both the environments in which the production was carried out and the molds involved in the process. The molds found in the different experiments are listed, with the respective authors of the studies, in Table 1.

For a correct interpretation of the data, it is crucial to add the table of Park et al. (1982), reproduced from Cereda and Brito (2018), which shows the distribution of the molds in three samples, and distinguishes the activity of alpha-amylase and amyloglucosidase (Table 2).

The identification of multiple molds, with irregular distribution in different observations, as the main agents of manioc saccharification, allows for some important considerations regarding the problem of the use of saccharification molds in the Amazon, as will be discussed in the analysis of the results. However, as noted by

Table 1. Amylolytic molds found in traditional *tiquira* production.

Reference	*Aspergillus*	*Neurospora*	*Paecilomyces*	*Penicillium*	*Rhizopus*
Maia (1949)	*A. niger*	*N. sitophila*		*P.* sp.	
Lima (1943)	*A. niger*	*N. sitophila*		*P. porpurogenum*	
Le Cointe ? cited in Lima (1975)	*A. niger*				
Lima (1975)	*A. niger*	*N. crassa*		*P. porpurogenum*	
Park et al. (1982)	*A. niger*	*N.* sp.	*P.* sp.		*R.* sp.
Chuzel & Cereda (1995)	*A. niger*	*N. sitophila*		*P.* sp.	
Venturini Filho & Mendes (2003)	*A. niger*, *A. oryzae*	*N. sitophila*			
Cereda and Costa (2008)	*A. niger*, *A. oryzae*	*N. sitophila*			
Ribeiro et al. (2011)	*A. niger*, *A. flavus*				*R. oryzae*

Table 2. Mold population in three samples of *beijus* collected in the state of Maranhão. Modified from Cereda and Brito (2018), based on Park et al. (1982). Legends: - = absent; CFU = colony forming units; * = in dextrin; ** = in glucose.

		Count (CFU/g)		Activity (Ul/g)	Activity (Ul/g)
Mold	*Beiju* 1	*Beiju* 2	*Beiju* 3	Alfa-amilase (*)	Amidoglucosidase (**)
Aspergilus niger	7. 10^5	6. 10^5	3.9. 10^5	-	1 497.00 ± 6.00
Paecilomyces sp.	1.1. 10^6	1.2. 10^6	-	57,00 ± 3,00	448.00 ± 2.00
Penicillium sp.	-	-	1.7. 10^5	-	-
Rhizopus sp.	1.5. 10^5	1.0. 10^5	1.8. 10^5	9,00 ± 1,00	1 276.00 ± 3.00
Neurospora sp.	1.9. 10^4	-	1.2. 10^4	-	452.00 ± 3.00

Cereda and Britto (2018, p. 477), it cannot be forgotten that these studies analyzed only the molds, which are no doubt important; however, in the process of fermentation, bacteria can enter, and in natural fermentations, in general, a succession of species occurs. The meaning of this statement can be better understood by analyzing the production process of *tarubá*.

TARUBÁ

Tarubá, or *tarupá*, is today a milky, sweet drink that can become alcoholic if left to ferment for longer periods. It is used in popular festivities, mainly in Santarém, Pará. It is obtained from grated manioc molded in *beijus*, which is left to rest for some days covered with leaves, and, when ready, diluted in water. Depending on the fermentation time, it can be a refreshing or alcoholic drink. It is considered an indigenous drink and is currently documented mainly among the *caboclo* populations. Frikel (1955, p. 225) considered it the drink of the Amazonian *caboclos*.

The main sources of documentation are relatively recent. Bates (1892 [1848-1859], pp. 221-246) finds it in Óbidos in 1849, and C. Souza (1873, p. 157) and Rodrigues (1875, p. 72) consider it an intoxicating drink; while the former does not express judgment, the latter considers it a disgusting drink. The two descriptions are similar, diverging only on the time during which the *beijus* are left to 'mold'. For Rodrigues (1875), it takes 3 days, whereas, for Souza (1873), it takes 7 days. The production process is identical to that followed for *tiquira* production, but the *must* is not allowed to ferment for more than 1 day. It becomes an alcoholic wine only when it is left to ferment for more than 3 days.

Stradelli's definition is "TARUPÁ' tarubá, *beiju* specifically prepared to make *caxiri*, from which the *tiquira* or manioc cachaça is extracted" (Stradelli, 2014 [1929], s.v.)[2]; it is, therefore, cassava *beiju* that is fermented to obtain a wine that is distilled to obtain *tiquira*.

Tarubá is also cited, with a similar description, by the great writers of Northern Brazil: Verissimo (1886, p. 88), Freyre (1950, p. 264), and Monteiro (1962, p. 65). Slow Food Brasil (2017) currently considers it a traditional drink, especially in the Santarém region.

In fact, *tarubá* is an intermediate product in producing a beverage using amylolytic fermentation before it is transformed into an alcoholic beverage. In amylolytic fermentation, the main transformation is represented, as the term indicates, by breaking down the starches that are transformed into simpler carbohydrates, fundamentally into sugars, through the saccharification of carbohydrates. Since the fermentation occurs with different microorganisms within a natural environment, the result will be a sweetened beverage with more or less acid depending on the prevalence of lactobacilli.

MICROFLORA IN *TARUBÁ*

Unlike what was verified in the case of *tiquira*, in the case of *tarubá*, it was possible to find only two complete analyses and one partial analysis. In theory, it can be expected that, since its preparation involves an initial production phase of the 'wine' that will be distilled into *tiquira*, the results of the analyses will be similar to those verified for *tiquira*. In reality, we find highly different results for two reasons: the analysis was performed for the fermentation liquid and not the *beijus*. Secondly, there are only two complete samples and one partial. The problem with *tarubá* is that the 'recipe' for its preparation can suffer local variations due to local tastes or traditions and thus present different results.

Carvalho (1998) found the domain of yeast, at the time defined *Hansenula anomala*, which has currently been redefined as *Pichia*, and a mold, *Rhizopus oligosporus*. The presence of *R. oligosporus* should not induce surprise because the same genus *Rhizopus* is also found in the production of *tiquira*. This species is used in Indonesia as a starter culture for soybean-fermented food (*tempeh*).

[2] "TARUPÁ' *tarubá*, *beiju* expressly prepared to make *caxiri*, from which the *tiquira* or cassava cachaça is extracted" (Stradelli, 2014 [1929], s.v.).

In the Carvalho (1998) test, *Rhizopus* stood out as the best producer of enzymes (total amylase and glucoamylase). *Hansenula anomala* (currently *Pichia*) presented more significant results in alpha-amylase production.

Sousa (2012) also identified only yeasts. *Pichia kudriavzevii*, *Pichia exigua*, and *Pichia membranifaciens* were the predominant species during the fermentation process of the drink. However, 232 microorganisms were identified in total, including filamentous fungi, which were not better identified, and were distributed according to Table 3.

The absence of amylolytic mold in the sample of Ramos et al. (2015) leaves us surprised. Dr. Rosane Freitas Schwan, who traced two of the three samples, stated that, during a visit to the Amazon, she observed two different preparation techniques: "some roll up [the cassava paste] on the banana leaf [that is left] completely closed, while others leave half open and always stir during the 12 days of fermentation" (Dr. Rosane Freitas Schwan, from Universidade Federal de Lavras, personal communication, 23/07/2020). However, since most molds are aerobic, their suppressed presence may depend on the type of wrapping with which the *beijus* are protected. However, as shown by Hesseltine et al. (1985), some molds, specifically those isolated from fermentation starters used for foods in Asian countries, may exhibit anaerobic growth. The three molds with these characteristics are *Mucor*, *Rhizopus*, and *Amylomyces* sp.; therefore, the presence of *Rhizopus* in the sample Carvalho (1998) would also be justified if the maturation phase of the *beijus* was anaerobic.

Table 3. Microbial population in Tarubá fermentation Modified from Oliveira (2012).

Microrganism	Number isolated
Yeasts	56
Lactic acid bacteria	61
Acetic acid bacteria	23
Gram positive bacteria	30
Gram-negative bacteria	36
Filamentous fungi (molds)	26
Total	232

The problem of conservation of *beijus* with aeration or total closure sheds light on a practice spread throughout the Amazon basin. Chewed or moistened cassava wrapped in banana leaves or other large leaves was traditionally transported as a food reserve.

DISCUSSION OF RESULTS

The results can be interpreted from two perspectives: from a strictly ethnographic point of view, to confirm the presence of amylolytic fermentation on the right side of the Amazon, and from the point of view of fermentation analysis, to better understand, based on a more significant number of biochemical analyses, the processes conducted in other regions, for which we have no data.

From an ethnographic point of view, the few observations confirm that amylolytic fermentation was conducted by populations living on the right side of the Solimões river, in Tefé and Amazonas state, and Santarém, Pará. The diffusion of *tiquira* into Maranhão, Piauí, and Alagoas suggests that amylolytic fermentation was perhaps also widespread among indigenous groups in these areas with a dryer climate but stable yearly temperatures (Bsk Hot semi-arid according to Köppen–Geiger classification). The presence of *tarubá* in the *caboclo* population represents further confirmation.

The most likely explanation for the low ethnographic documentation among indigenous populations is that the right side of the Amazon suffered a more substantial impact in the early days of European missionary and extractivist activities at the beginning of the Colonial period. The riverside populations were more rapidly 'reduced', but their cultural practices have survived today in *caboclo* communities. As written by Balée (1995), sedentary populations who became nomadic when fleeing the colonizers chose corn over cassava because it grows faster. Thus, the tradition of amylolytic fermentation among indigenous groups may have been lost.

Outside the Amazon basin, the environmental conditions of lower humidity and fluctuations in temperature probably did not encourage this type of processing.

In the ethnographic view, one more possible piece of evidence in the account of Acuña (1891 [1641]) would extend the spread of this type of fermentation. The chronicler, describing life on the islands of the Omaguas, wrote that when the islands were flooded, the natives resorted to two forms of storage (Acuña, 1891 [1641], p. 25). In the first, manioc tubers were buried before the beginning of the flood, and when the waters descended, they could be consumed without losing value. It was, therefore, a typical 'pit storage' conservation process, similar to that used in Polynesia to conserve breadfruit and bananas. However, with this method, manioc became available only when the flood waters were low. The other method (Acuña, 1891 [1641], p. 23), on the contrary, allowed the immediate use of manioc; *beijus* were made and stored in the high parts of *malocas* (indigenous houses) for long periods and could be crumbled in water and boiled, producing the wine that was usually drunk.

In the current process of *tiquira* production, the *beijus*, after having suffered the saccharification of starches, can be immersed in water to be fermented or stored. The formation of molds during the saccharification process likely protects them from other aggressive forms of breakdown (by unwanted molds). On the other hand, the native tradition of the Amazon basin is the daily processing of manioc. The status of the Omaguas was naturally different; with the islands periodically flooded, it was necessary to find a way to conserve food during the flood season, and this mechanism was found in the conservation of the *beijus*, which I think could have been moldy. In fact, Acuña does not mention that the cassava paste should be chewed. Markham (1859, p. 66), in his edition of Acuña, mentions a description of how, in the Amazon, chewing was used to make fermented beverages, which Acuña does not mention. However, this is only a hypothesis awaiting confirmation.

The results are much richer when used to interpret the occurrences of fermentation in the rest of the Amazon, for which there is just one biological analysis.

The great variety of molds found simultaneously and in different samples suggests that the process does not occur with a single component, as in a laboratory, but with multiple elements. Microbial flora can probably differ over time, depending on various variables such as season, weather conditions, temperature, air humidity, and the environment from which the cassava originates. Park et al. (1982) and Ribeiro et al. (2011) showed that the varieties of mold are not neutral in relation to the result and that, therefore, in the laboratory, an optimization of the process is possible; however, in a natural context, the tendency is to maintain the variety, depending on environmental conditions. This phenomenon is also common in the East. As reported by Boulton and Quain (2001, p. 28), in Japan, *saké* is produced with a single mold, *Aspergillus oryzae*, whereas "Chinese saki is prepared using a microbial flora . . . . develops spontaneously and contains yeasts as well as various" molds "belonging to the genera *Rhizopus*, *Mucor*, *Penicillium*, *Absidia*, and *Monascus*, in addition to *A. oryzae*" (Boulton & Quain, 2001, p. 28).

The presence of *Aspergillus niger*, *oryzae*, and *flavus* confirms what was advanced in a previous study: the possibility that this mold was present in other fermentations since it is present in many oriental products and is a verified contaminant in cassava byproducts in Africa and Southeast Asia, in regions with similar climatic conditions. Its high saccharification activity justifies its presence in almost all samples, as it is the most effective mold in the process. The confirmation that the process described in a previous article on Miskitos (Conzemius, 1932) is attributable to *Aspergillus* was corroborated by Sandor Katz (personal communication, 08/24/2020). He informed that amylolytic fermentation of corn is still in use among the Bribri, an indigenous group on the Caribbean coast. They made *chicha* using a molded starter called *oko* in Bribri, and *mohoso* in Spanish, prepared by cooking and grinding corn,

wrapping the mash in banana leaves, and allowing the mass to mold spontaneously. Without laboratory analysis, Katz recognized that the main mold was *Aspergillus* sp., but other molds appeared in the paste.

The presence of *Rhizopus* sp. confirms what was verified by Henkel (2004) among Wapisiana. Since Henkel identified only *Rhizopus*, the process described, with the use of the same leaves as in the previous fermentations, probably functioned as the source of the inoculum to maintain the contamination of the *beijus*. On the other hand, Park et al. (1982) showed that *Rhizopus* is not the most efficient mold for the saccharification of cassava starches.

New data include *Neurospora sitophila*, which was not considered in the previous study. Still, it deserves maximum attention because it probably explains some of the procedures followed in other indigenous fermentations. It is essential to mention that *N. sitophila* is used worldwide to describe a single food, *Oncon* (Sastraatmadja et al., 2002), produced in Indonesia by fermenting the scraps of tofu or peanut pie, from which the oil has been removed. Sometimes, manioc pulp or tapioca is added to these two ingredients.

A search of the natural history of this mold allows a better understanding of the meaning of its use. It mainly offers a clear indication of the possible widespread use in producing Amazonian fermented beverages.

Neurospora is a family of saprotrophic molds that feed on decomposing material; however, one of its characteristics is that it is also a pyrophilic fungus, whose development is stimulated by fire (Turner et al., 2001), developing on burnt tree branches. Ascospores of *Neurospora* remain dormant until exposed to moist heat above 65 °C, i.e., temperatures in boreal forests only occur during fires (Perkins et al., 1975). "After a fire, *Neurospora* germinate and form a characteristic 'orange bloom' on the black, charred tree trunks" (Stivrins et al., 2019, p. 1480).

Another characteristic of *N. sitophila* is that it emanates a fruity aroma in the fermentation process (Yoshizawa et al., 1988; Turner, 1992), which is attributed to the compound ethyl hexanoate.

We compared these two characteristics of this mold with Maroni's descriptions of the amylolytic fermentations in the upper Amazon (Maroni, 1889 [1738], pp. 135-136).

> [the beverage] they all have for the most noble events and use in the most solemn feasts is the Chaburaza, which the Napos, to ennoble it, call the soup of wine. It is made in this way: the manioc is peeled and cooked in a large pot with little water (the Napos and others toast them in a smokehouse without removing the peel). Then, they put manioc roots on the floor or barbecue them on clean banana leaves. On the second day, they are sprinkled with a colored powder, which is the mold that grows on the sticks of the farm or on roots of the same manioc, half burned, and covered with other banana leaves. [They are left] for four days, in which time they are covered by that powder and breed something like a bloom of the same color, and this is what gives the Chaburaza a sweet and flavorful taste, so without that powder it is usually tasteless (Maroni, 1889 [1738], pp. 135-136, added emphasis).

The two characteristics, marked in bold in the quoted text, allow, confidently, to state that the amylolytic mold used was *N. sitophila*. Osculati's text offers a similar impression:

> During this process, they mix the dough with a red powder or flour, which they artificially obtain by cutting into small pieces the stem of a yuca-brava [bitter manioc] shrub that they toast and leave to the dew for eight days. At the end of this period, a mold of a beautiful scarlet color appears on the stem, which has a good smell and comes off at the slightest puff of air. This powder has the property of fermenting and souring the banana dough that is left wrapped in those large leaves for a certain time, after which the preparation is completed (Osculati, 1854 [1846-1848], pp. 111-112, added emphasis).

Osculati (1854 [1846-1848]) also mentions the two characteristics of *N. sitophila*: it is formed in burnt vegetal material and has a pleasant odor. The only divergence from the mold characteristics is the color, red, according to Moroni, and scarlet, according to Osculati, defined as orange or salmon (Stivrins et al., 2019; Hsiao-Che et al., 2014). However, it *must* be remembered that when the first species of *Neurospora* was discovered almost two centuries ago in a Parisian bakery, it was defined as

champignons rouges, and named *Monila sitophila* (Roche et al., 2014, p. 2022).

Joshua Homan, from Universidad San Francisco de Quito (USFQ) (personal communication, 09/08/2020) confirmed that amylolytic fermentation is still occurring in the upper Amazon, among the Kichwa Canelos of Bobonaza, Achuar, Shiwiar, and Zaparo villages. The process has been documented by Whitten (1976, p. 88) and Reeve (1985, p. 151). Whitten's description follows: "An orange powder-like fungus (probably *neurospora*) is sprinkled with the tubers. Orange, white, black, and yellow fungi develop in the tubers, and within ten days, everything inside the skin has succumbed to a bright orange growth"[3].

In the case of *tarubá*, it is important to try to understand what it was in reality. By Stradelli's definition, *tarubá* was "*beiju* expressly prepared to make the *caxiri*, from where the *tiquira* or manioc cachaça is extracted" (Stradelli, 2014 [1928], s. v.). By metaphor, it becomes the drink's name obtained by crumbling the same *beiju* into the water, without a subsequent fermentation. In practice, as indicated by other chroniclers (Maroni, 1889 [1738]; Daniel, 2004 [1750]; C. Souza, 1875), since the *beiju* becomes sweet after amylolytic fermentation, the process would result in a sweet drink which is both thirst-quenching and is slightly alcoholic. It can be expected, therefore, that the results of the biological analyses would be similar to those obtained in the tests with *tiquira,* but this does not occur. The reasons for this could be multiple. The first is that, without the need to produce alcohol, which is the essential purpose of *tiquira* production, the processes do not have feedback; hence, they tend to develop seeking a flavor, which, depending on preferences, can be more or less sweet or acidic. The second is that the sampling takes place in a *caboclo*, rather than an indigenous environment; therefore, it currently does not necessarily follow the processing pointed out by observers of the 19th and early 20th centuries.

The analysis of the biochemical characteristics of the preparations confirmed outside of laboratories shows that fermentation results can be strongly variable, as already shown in relation to *tiquira*. However, a striking feature of the microflora in *tarubá* is the predominance of lactobacilli and the absence or limited amount of filamentous fungi with amylolytic properties. Dr. Schwan's comment that in an amylolytic fermentation process, the material may be more or less exposed to aerobic fermentation introduces a critical variable.

The problem of conservation of *beijus* with aeration or total closure sheds light on a practice spread throughout the Amazon basin. Wet or chewed manioc wrapped in banana leaves was generally transported as food during long trips or hunts. This practice has been reported by many travelers, although most mentions were made during long voyages and expeditions. While the geographic area might be provided, there is often ambiguity regarding the ethnic group involved (Grillet & Bechamel, 1698 [1674], p. 52 - Guiana; Osculati, 1854 [1846-1848], pp. 186-187 - Napo river; Schomburgk, 1931 [1835-1839], p. 1 - Guianas; Koch-Grünberg, 1921 [1905], p. 39 - Rio Negro; Métraux, 1942, p. 73 - Eastern Bolivia; Pierre, 1889, p. 52 - Ecuadorian Amazon). The cassava mass transported over long journeys, sometimes buried or hidden in holes of trees to be used on the return journey, was the reserve of food diluted in water to make a porridge. Another practice was burying food in pits for long-term conservation (Acuña, 1891 [1641], p. 22).

In all these cases, due to anaerobic conditions, fermentation probably developed similar to *tarubá* production. In this sense, the results of the *tarubá* analysis can be considered an anticipation of what can be expected from tests with other types of aerobic fermentation. By analyzing starch grains from archaeological finds, archaeologists are today beginning to recognize some of these practices narrated by chroniclers and confirmed

[3] The use of a red mold is confirmed, among the Yorimagua, in Marañon River and Morona Santiago Rivers by the Jesuit Chantre y Herrera (1901 [1767], p. 85), and in a quichua community by Gallegos (1997, p. 180).

by indigenous and popular traditions, which went into producing so-called 'Indian bread' (Santos et al., 2021). To fully understand the processes behind Indian bread (*'pão-de-índio'*), it will be important to perform field tests, but the tradition of pit storage, widely used in Polynesia to store fruit from the bread and banana trees (Steinkraus, 1996, p. 309; Battcock & Azam-Ali, 1998, p. 53), may indicate the importance of lactic fermentation in its fabrication, a process also associated with European sauerkraut.

CONCLUSIONS

A study of two fermentation processes currently prevalent in *caboclo* but not among indigenous populations provided new information on historic fermentation processes. It increased the knowledge about the number of amylolytic molds used. It especially confirmed the presence of this type of fermentation also on the right side of the Amazon.

This fact should not be surprising. A warm, humid climate characterizes the Amazon basin. In these environments, food conservation represents a challenge; molds and yeasts alter any food in a few hours, and insects devour what is left over. It is not surprising that food preparation is daily because what is left over will not be edible the next day. But *farinha* and *beijus* may be stored for a long time. It is in this context that Amazonian civilizations developed. The fermentation of food is one of the few ways to ensure conservation.

Perhaps the best way to visualize the difference in food preparation between the humid forest and the coastal Tupi, which have a drier, more seasonal climate, is to observe food prepared for hunting or war expeditions. On the Brazilian coast, the food carried during expeditions was '*farinha de guerra*' or war flour, a very dry cassava flour (G. Souza, 1879 [1587], p. 158; Brandão, 2010 [1618], p. 208; Gandavo, 2008 [1570]; a comprehensive review in Cruz 2011), often added to dry fish flour. In the Amazon forest, the food carried was moistened cassava, often partially chewed and wrapped in leaves during fermentation. Only with a product in the fermentation phase, in which the fermentation environment has eliminated contamination, can the food be preserved during a voyage without undergoing deterioration.

Today, due to the tests performed on an industrialized product, we can begin to better understand what was probably the more complex fermentation process that indigenous populations had developed before European conquest: the saccharification of starches with amylolytic molds. We also have good information about the fermentation processes of carbohydrates that follow chewing. On the contrary, there are no studies on the conservation processes of moist foods that are transported wrapped in vegetable packages and on conservation by pit storage, which were probably essential practices in all fermentation processes. A complete understanding of these processes is essential to fully appreciate the processes of food preparation and conservation among Amazonian cultures.

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