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Using Purple Amerindian Yam (cará roxo, *Dioscorea trifida* L.) as brewing adjunct: technical and sensorial analysis

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Abstract

Purple Amerindian Yam (PAY, Dioscorea trifida L.) is an important component of traditional people dietary in Brazilian central amazon rainforest. It is mainly produced by family farmers, using ecologically friendly ways of cultivation. Face to the new Brazilian legislation about brewing adjuncts, its technical viability for use as brewing adjunct was evaluated, besides a sensorial analysis of the resulting beers by trained consumers. Were produced 4 different beers: control group (only barley malt), PAY15, PAY30 and PAY45, using 15%, 30% and 45% of PAY in substitution of barley malt respectively. Along mashing phase, sugar concentration was affected only in PAY45, while soluble protein increased in all assays using adjunct. The kinetic of fermentation was not affected in any assay. In the final products, not significant differences were observed for pH and sugars and proteins concentration. Alcoholic concentration was signiticantly decreased only by the PAY45 assay. EBC scale values increased in assays using PAY, due its purple color. Flavors and aroma's perceptions for hops and ferment were not affected, being the only significant organoleptic difference observed to malt's flavor in PAY45 assay. These results indicate the viability of PAY as brewing adjunct, viable to substitute until 30% of barley malt.

Keywords: Purple Amerindian Yam; brewing adjunct; artisanal beer.

Practical Application: The use of *Dioscorea trifida* L. as brewing adjunct will foment a local productive chain and improve the income of family farmers.

1 Introduction

Purple Amerindian Yam (PAY), in Portuguese named "cará roxo", is a starchy root of the family Dioscoreacea, and one of the ten species of this family important in human nutrition. According to Lebot (2009), *Dioscorea trifida* L. was domesticated by pre-Columbian people, and probably was the first yam cultivated by indigenous and immigrants in central Amazonia. Currently, PAY is cultivated by family farmers, produced by ecological friendly ways in Amazon basin river's floodplain and used for scholar feeding in all Amazonas state, Brazil.

In other hand, artisanal beer is a growing market niche in Brazil, being in 2018 a total of 889 breweries registered in Ministry of Agriculture. Along 2020, 30 breweries canceled their registers, probably due economic crisis. Despite of this, there were registered 204 new breweries, and currently, the total number of breweries registered in Brazil is 1383 (Brasil, 2021). It has occurred mainly because the simplification of the process for registration and granting of operating license (Marcusso & Müller, 2019). Furthermore, the legislation about beer production was recently modified.

Previously, Brazilian legislation restricted beer as "the drink obtained by alcoholic fermentation of brewing wort from barley malt and potable water, by action of the yeast, with addition of hops" (Brasil, 2009). Currently, new legislation stablishes that "beer is the drink resulting from fermentation, by brewing yeast, of barley malted wort or malt extract, previously submitted to cooking process added of hops or hop extract, in this hypothesis a part of the barley malted or of the malt extract may to be partially substituted by brewing adjunct" (Brasil, 2019). The most important obligation for the brewery is informing to the consumers what adjuncts and other ingredients were used in the productive process.

Face to the possibility of using alternative starchy as brewing adjunct and the increasing market niche of artisanal beer, the aim of this work was to evaluate the viability of using PAY as brewing adjunct, followed by analysis of its organoleptic properties by trained consumers. This use can improve the income of family agriculture in amazon basin and create an environmentally friendly productive chain.

2 Material and methods

2.1 Barley malt, yeast and hops

In this research, all experiments were performed using Pilsen barley malt by Best Malz® (Germany), yeast *Saccharomyces cerevisiae* strain Safale US-05 (*Ale* fermenter) by Fermentis® (France) and hops US Brewer Gold T-90 (6,9 percent of alpha-acids) in pellets by Eureka Brewery Supplies (USA).

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2.2 Brewing protocols

Were evaluated three different protocols of brewing using PAY as adjunct, and a standard protocol was performed as control group, according to Table 1. The protocols were performed in low scale (250 mL Erlenmeyer flasks) and in expanded scale (20 L fermenter vessels).

The protocols PAY15, PAY30 and PAY45 used Purple Amerindian Yam as brewing adjunct substituting respectively 15, 30 and 45 percent of the barley malt. In all cases, the mashing phase was initiated adding the barley malt and the adjunct, when present, to a half of the water at 68 °C. Then, the temperature was maintained at 65 °C for 80 minutes, elevated to 76 °C and maintained for 20 minutes. The liquid was transferred to another vessel, and the second half of the water (at 76 °C) was used to wash the spent grains, resulting in the brewing wort.

The brewing wort was boiled for 60 minutes, being added a first half of hops when the boiling has started. Last 10 minutes of this phase, second half of hops and a flocculant tablet (carrageenan, 0.025 $g.L^{-1}$) were added. After the hops waste was sedimented, the liquid phase was cooled at 23 °C and transferred to a fermenting vessel. The yeast was inoculated (about 0.500 $g.L^{-1}$) and the vessel closed with a water seal to allow the carbon dioxide releasing.

To primary fermentation phase, the vessel was maintained at 21 °C for 7 days, and more 7 days at 4 °C for maturation (secondary fermentation). The low scale assays were interrupted and used to evaluate the responses variables. The expanded scale assays were added of caramelized crystal sugar (4.0 $g.L^{-1}$), distributed in sterilized bottles (about 600 mL per bottle) and sealed using metal stoppers. The bottles were maintained at 21 °C for 7 days for carbonation / gasification (final fermentation), being cooled at 4 °C after this time.

2.3 Response variables

In low scale assays, ethanol concentration (%ABV) was calculated based on CO_2 mass released, estimated by stoichiometric calculation using the formula "$mEtOH = mCO_2 * 1.045$", according to Van Dijck et al. (2000). The kinetics of fermentation was characterized by specific growth rate (μ_{MAX}, h^{-1}) of ethanol concentration, calculated according to the formula "$y = \mu_{MAX} * x \pm b$", being "$y = \ln (mEtOH_n / mEtOH_0)$" along exponential phase of fermentation.

In expanded scale assays, ethanol concentration was calculated according to Papazian (1984) using the formula "$\%ABV = \Delta d * 131.25$", being %ABV the percentage of alcohol by volume and Δd the difference between initial and final density.

To evaluate the effects of PAY addition, total reducing sugar concentration ([TRS], $g.L^{-1}$), total soluble protein concentration ([TSP], $g.L^{-1}$) and density were evaluated after mashing, primary fermentation and carbonation phases.

The color of each beer was classified in European Brewing Convention (EBC) scale, determined by measuring of the absorbance at 430 nm (A_{430}), using distilled water as blank. The EBC scale value was calculated using the formula "$EBC = A_{430} * 25$" according to Smythe & Bamforth (2000).

2.4 Sensorial analysis

Aiming to evaluate organoleptic characteristics, as well the interference of PAY in flavors and aromas, six trained consumers, both genders and with ages between 22 and 45 years old, were invited to taste the beers using a blind tasting method. Flavors and aromas were classified according to Strong & England (2015). They were required to answer an informed consent form, being this a criteria of exclusion.

Four bottles, each one containing about 600 mL of each beer produced according Table 1, were provided to each taster. The only difference among each sample was a colored decorative twine on the bottleneck, as presented at Figure 1.

They're asked to answer an electronic formulary (available in supplementary material) telling comparatively how much capable they were to percept flavors and aromas of hops, malt and ferment in each beer, assigning a score from 0 to 10, being "zero" the absence and "ten" the maximum perception of flavor and aroma. The scores assigned to each brewing protocol were compared to the scores assigned to control protocol.

2.5 Analytical methods and Statistical analysis

Total reducing sugar ([TRS], $g.L^{-1}$) was evaluated using DNS method, as previous described by Magalhães et al. (2018). Total soluble protein ([TSP], $g.L^{-1}$) was evaluated using Biuret method,

Figure 1. Bottles provided to the consumers, being the only difference, among these, the colors of the decorative twines on the bottle-neck. Yellow twine bottle contains the control protocol beer, while blue, green and red twine contains the beers made using PAY15, PAY30 and PAY45 protocols, respectively.

Table 1. Experimental design of brewing protocols using purple Amerindian yam and control group.

	Barley malt ($g.L^{-1}$)	Purple Amerindian Yam ($g.L^{-1}$)	Hops ($g.L^{-1}$)
Control group	130.0	-	1.5
PAY15	110.5	19.5	1.5
PAY30	91.0	39.0	1.5
PAY45	71.5	58.5	1.5

according to Keppy & Allen (2009). Density was measured using a densimeter, and Brix degree was measured using a portable refractometer. All analysis were performed in triplicate. All quantitative results were evaluated by Mann-Whitney U test ($\alpha = 0.05$) using JASP® (version 0.14.1.0).

3 Results and discussion

3.1 Low scale assays

Using of PAY led to a reduction in [TRS] in the brewing wort, besides increasing it [TPS], being observed significant difference as for [TRS] as for [TPS] ($p = 0.049$ and $p = 0.034$, respectively). Total reducing sugar and total soluble protein concentrations averages on brewing wort are presented at Table 2.

The experiments performed in Erlenmeyer flasks results in final ethanol concentration on average of 44.5 $g.L^{-1}$ (4.45%ABV) in control assays, and 47.8, 36.0 and 29.7 $g.L^{-1}$ for PAY15, PAY30 and PAY45, respectively. The variation of ethanol concentration in all assays along fermentation phase is presented in Figure 2.

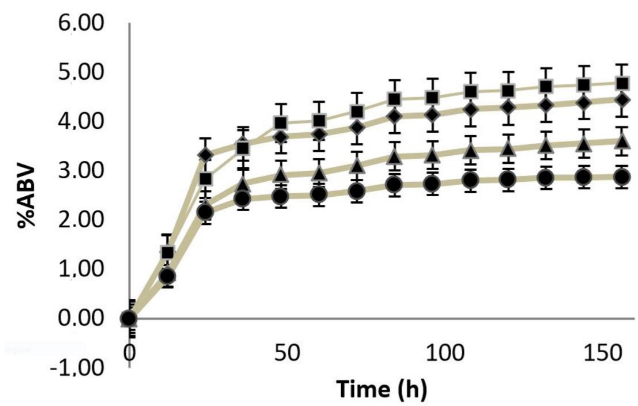

Figure 2. Variation of ethanol concentration (expressed in %ABV) along primary fermentation phase in control (♦), PAY15 (■), PAY30 (▲) and PAY45 (●) assays.

Table 2. Average values of total reducing sugar concentration ([TRS]) and total soluble protein ([TSP]) in brewing wort. The * indicates significant difference values.

Assay	[TRS] $g.L^{-1}$	[TSP] $g.L^{-1}$
Control	43.41 (±4.80)	8.86* (±1.22)
PAY15	33.22 (±5.09)	11.63 (±1.43)
PAY30	33.48 (±4.60)	10.81 (±0.82)
PAY45	23.88* (±3.68)	10.61 (±0.78)

When compared to control group, final ethanol concentration difference of PAY15 and PAY30 were not significant ($p = 0.827$ and $p = 0.275$, respectively), being observed significant difference only for PAY45 ($p= 0.049$). This result indicates that using PAY as brewing adjunct, substituting until 30 percent of barley malt, does not significantly affects the final ethanol concentration.

Considering the exponential phase between 12 and 60 hours, the specific growth rate was calculated only along this time interval. The values of μ_{MAX} were, on average, 0.017 h^{-1} for control group, and 0.021, 0.019 and 0.019 h^{-1} for PAY15, PAY30 and PAY45 assays, respectively. Statistical analysis indicates not significant difference ($p = 0.363$), meaning that PAY addition does not affect the kinetic of fermentation.

3.2 Expanded scale assays

The expanded scale assays allowed the analysis of the response variables at the final product, being evaluated the effectiveness of the values predicted in low scale assays.

Total reducing sugar and total soluble protein in the brewing wort presented the same feature observed in low scale assays. The only significant difference of [TRS] was observed to PAY45 ($p = 0.029$), while [TSP] was significantly lower in control assay ($p = 0.006$). In the final product, [TRS] and [TSP] averages were 8.74 and 4.68 $g.L^{-1}$, respectively, with not significant difference. Final values of [TSP] indicates the consumption of these nutrients along the fermentation. Complete results are presented on Table 3.

Final products obtained by each brewing protocol presented on average alcoholic concentration varying among 2.67 and 4.78% of alcohol by volume. The final pH was not different when comparing each beer, with average of 4.30.

The values of the EBC scale were variable, presenting since 9 (yellow) in control assays reaching until 33 (deep copper) in PAY45 assays. This result was expected because of the purple color of the adjunct. The color was not a stable response variable, occurring colors around light copper (EBC 22) in some assays using PAY45 protocol. It occurred because of variable intensity of the purple color in different samples of PAY. Complete results of each beer are presented at Table 4.

The use of PAY as brewing adjunct results in a beer with alcoholic concentration significantly lower ($p = 0.049$) while pH had not significant difference. Furthermore, the use of PAY adds color to the beer, increasing significantly ($p = 0.016$) the EBC scale value in the protocols with greater ratio of adjunct.

Table 3. Average values of total reducing sugar concentration ([TRS]) and total soluble protein ([TSP]) in brewing wort and in final product (beer).

Assay	Brewing wort		Final product	
	[TRS] $g.L^{-1}$	[TSP] $g.L^{-1}$	[TRS] $g.L^{-1}$	[TSP] $g.L^{-1}$
Control	41.40	21.33^{b}	9.54	4.62
PAY15	35.45	27.69	8.05	3.83
PAY30	29.88	28.09	9.75	4.57
PAY45	26.75^{a}	25.41	7.64	3.58

Subtitle: ^{a} and ^{b} means significant difference (p-value 0.029 and 0.006, respectively).

Table 4. Characteristics of each beer obtained according to the brewing protocol.

Assay	Characteristic			
	Original density average	%ABV average	pH average	Color (EBC value) range
Control	1.041 (±0.003)	4.24 (±0.54)	4.32 (±0.08)	9 - 17
PAY15	1.038 (±0.004)	3.99 (±0.59)	4.28 (±0.06)	9 - 14
PAY30	1.037 (±0.003)	3.74 (±0.75)	4.30 (±0.08)	15 - 23
PAY45	1.031 (±0.002)	2.83 (±0.16)	4.28 (±0.04)	22 - 33

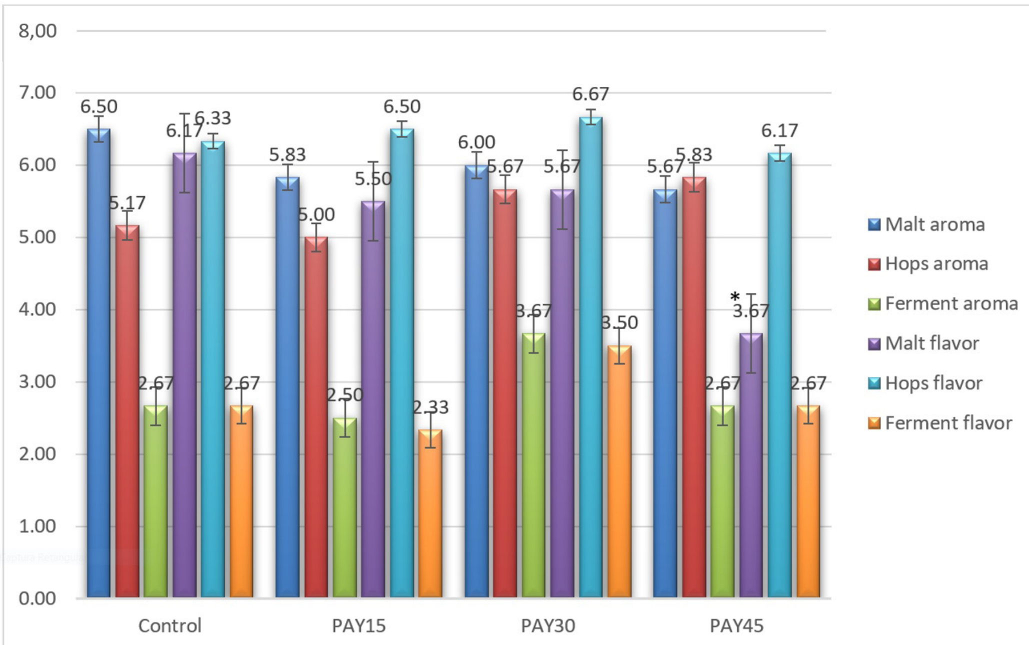

Figure 3. Scores average for perceptions of aromas and flavors of malt, hops and ferment. The * indicates significant difference (p = 0.043).

3.3 Sensorial analysis

There was not significant difference in the perceptions of aromas, neither of hops and ferment's flavors. The only one significant difference (p = 0.043) was perceived in malt's flavor, with score about 60% of the control group. Complete results of sensorial analysis are presented in Figure 3.

4 Discussion

Up to 85-90% of the beer produced around the world uses adjuncts, mainly because of difficult to cultivating barley and to reduce the costs of production. In most of the cases, the brewing adjuncts support and reflect the local agricultural market. In Africa, for example, sorghum is the most common adjunct, while rice and corn are the most common in Asia and America, respectively (Bogdan & Kordialik-Bogacka, 2017). Some uncommon adjuncts had been tested like faba bean (Black et al., 2019), buckwheat (Deng et al., 2019) and pine tree seeds (Jorge et al., 2018).

The brewing wort obtained by using until 30% of PAY as adjunct presents similar original density with that using faba bean's flour, with the advantage that Black et al. (2019) used exogenous amylases to reach this result. It indicates that is possible to use until 30% of PAY without reduce significantly the enzymatic efficiency of the barley malt. Furthermore, PAY presents advantage as brewing adjunct when compared to pine tree seeds (in Portuguese named "pinhão") flour. According to Jorge et al. (2018), when using "pinhão" flour over 20%, exogenous amylases are recommended to avoid enzymatic efficiency loss. The lower %ABV observed when using PAY45 protocol is due to the lower original density and reducing sugar concentration of the brewing wort.

While other adjuncts as corn, sorghum, unmalted barley and husked oat, decrease the total soluble protein (Agu 2002; Schnitzenbaumer & Arendt, 2014), the use of PAY increases significantly the soluble protein concentration in the brewing wort. This fact can explain why does the kinetic of the fermentation was not affected in this work, evidenced by the lower [TSP] in the final product.

When using sorghum as brewing adjunct, Schnitzenbaumer et al. (2013) observed an increasing in final product's pH, similarly to the faba beans flour's beer (Black et al., 2019). The pH of beers PAY15, PAY30 and PAY45 didn't present significant variation, reinforcing the potential of this root as brewing adjunct without requiring corrections of pH in the final product.

The only significant difference perceived by the sensorial analysis was lower flavor of malt in the beer obtained by protocol

PAY45. Uncommon adjuncts can interfere in the beer acceptance. Consumers reported significant interference in the flavor when “pinhão” was used as brewing adjunct, assigning lower acquisition’s intention when compared to control group (Batista, 2014). Blind sensorial analysis indicated not interference of PAY, as for flavors as for aromas, when substituting until 30% of the barley malt. This result is similar to the observed for the craft beer produced with 30% of faba beans (Black et al., 2019). These facts reassure the viability of PAY as brewing adjunct, mainly when used until 30%, not interfering in sensorial characteristics of the beer. Additional methods must to be included, including a risk perception (Silva et al., 2021), in a further sensorial analysis with a greater sample of consumers, aiming to evaluate the acceptability of this artisanal beer.

There are different motivations for choosing a brewing adjunct, but most of these are for costs decrease (Rosentrater & Evers, 2018). Purple Amerindian yam is produced seasonally by family farmers, small-owners, in central Amazon rainforest using rudimentary technologies and environmentally friendly ways of cultivation. The choice of purple Amerindian yam as brewing adjunct to an “Amazonian artisanal beer” can improve the income of family farmers, decrease the waste of this food and to foment a local productive chain.

More than to propose a new brewing adjunct, this work proposes the improvement of a sustainable productive chain for small-owners in central Amazon rainforest. Due to the tendency of Brazilian consumers for innovativeness (Delorme et al., 2021), *Dioscorea trifida* L. presents, beside technical viability, high potential of market as a new uncommon brewing adjunct.

5 Conclusions

Purple Amerindian yam is a viable brewing adjunct, being appliable to substitute until 30% of barley malt without affect the enzymatic efficiency along mashing phase neither the kinetic of the fermentative process.

In the final product, pH, and perceptions of aroma and flavors of hops and ferment were not affected. Beside of this, its use adds color to the beer, increasing significantly the values in the EBC scale.

The %ABV and malt’s flavor were significantly affected only when using 45 percent of PAY, as expected.

The subsequent efforts will be employed to evaluate, besides risk perception about alcoholic beverage, the acceptance of PAY15 and PAY30 beers by untrained consumers, to assessing the market potential of this product.

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