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Maceration of acai (*Euterpe precatoria*) in alcohol vinegar as a strategy to add colour and functional compounds to the acetic fermentate

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Abstract

*The interest in daily consumption of healthy products has increased considerably in the last years mainly due to the increase aware of the relationship between food and disease prevention. Acai, a native fruit of the Amazon, has a high concentration of functional compounds and known worldwide as a superfruit. The objective of this work was to add colour and functional compounds of acai berries to alcohol vinegar by macerating the fruit for 7, 14 and 21 days using different proportions of alcohol vinegar: acai (1: 1 and 9: 1). The vinegar used was supplied by Virrosas manufacturer and the acai was purchased from a local producer. After maceration, the vinegars were subjected to analysis of total dry extract (TDE), ashes content and colour determination by direct reading of the coordinates L *, a *, b *, C * and h° with the DeltaVista spectrophotometer in the time zero and after 18-month storage in an amber glass container kept at room temperature. For almost all analysis performed, a marked difference*

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was observed between both proportions of alcohol vinegar: acai employed, with higher values for the 1:1 ratio when compared to 9:1. Also, the maceration time interfere in the results obtained and in general no marked differences were observed between 14 days and 21 days of maceration, indicating that the maceration should be carried out for a maximum of 14 days. The results showed that acai compounds were transferred to alcohol vinegar and future work will be performed to identify these compounds.

Key words: acai, maceration process, alcohol vinegar, colour, ash content, total dry extract

INTRODUCTION

The vinegar is produced by a double fermentative process, an alcoholic fermentation followed by an acetic fermentation, in which the ethanol is oxidized and transformed into acetic acid by acetic bacteria. The vinegar can be obtained from different raw materials and its origin is related to differences in chemical composition, nutrients and flavors. The chemical and organoleptic properties of vinegar are determined by the acetification system used, the raw material used as a substrate and, in some cases by the aging time in wood (Ho et al. 2017). The vinegars can be made from red or white wine, alcohol, brandy and fermented products obtained from different fruits. They can also be made from starchy raw materials, such as potatoes, sweet potatoes and cassava, which produce the so-called starchy tuber vinegars, sugary materials like honey, molasses and sugar syrups and from cereals such as barley, rye, wheat, corn and rice (Palma et al. 2001).

In Brazil, the general culture does not value the quality of the vinegars consumed, what determines the low prices of the product on the market. The consumption of fruit vinegars is very small, the main raw material being sugarcane ethanol. According Zilioli (2011), this fact probably can be related to the lack of technical knowledge of the manufacturing process by the producers, the use of very cheap raw materials, little publicity of the product, consumer's ignorance of its

functional properties, with its possible uses and applications, and this is also a reflection of the small number of researches carried out in the country on this topic.

Fruit vinegars are increasingly popular around the world and the consumption of so-called special vinegars has grown in comparison with other commercial vinegars, whose market is stationary. More and more products are being sought with greater added value, with better sensory attributes and proven functional capacity (Coelho et al. 2017). The biological properties of vinegars, especially fruit vinegars, have been the subject of researches in recent years, in search of deepening on the subject, even though the product has had medicinal uses for centuries. The beneficial effects of vinegar are due to various types of polyphenols, micronutrients and other bioactive compounds found in vinegars that contribute to the pharmacological effects, including antimicrobial, antidiabetic, antioxidant, anti-obesity and anti-hypertensive (Sakanaka & Ishihara 2008; Ho et al. 2017). According to BASTANTE et al. (2010), the process of maceration of fruits or fruit peels in vinegars could contribute to the development of a final derivative of vinegar with beneficial effects for the consumers health.

This work was carried out with the objective of evaluating the maceration of acai in alcohol vinegar produced by a local manufacturer in other to add colour and the functional compounds of acai berry to alcohol vinegar by macerating the berries for 7, 14 and 21 days using different proportions of alcohol vinegar: acai (1: 1 and 9: 1) and to elaborate an innovative food product from a regional fruit.

MATERIALS AND METHODS

All experiments were carried out at the Food Technology Laboratory of Campus Manaus Centro, IFAM.

Materials and Design of Experiment

The alcohol vinegar used was supplied by the company Virrosas and the acai was purchased from a local producer. The experiments were carried out considering two parameters, the proportion alcohol

vinegar: acai and the maceration time. Glass bottles with a capacity of 500 mL, previously sterilized, each containing alcohol vinegar: acai in the proportions 9: 1 and 1: 1 were macerated for 7, 14 and 21 days (Figure 1).

Figure 1. Beginning of the acai maceration process in alcohol vinegar

All experiments were carried out in triplicate. After the maceration time, the vinegars were subjected to physical-chemical analysis of total dry extract, ash content and colour parameters. The colour parameters were measured at time zero and after 18 months storage in an amber glass container kept at room temperature.

METHODS

Determination of Total Dry Extract (TDE)

The TDE was determined using the gravimetric method and is based on the quantification of the extract after evaporation of water and volatile substances. It was pipetted 20 mL of the sample in an aluminium capsule, previously tared, followed by evaporation in a water bath until syrupy consistency and drying in an oven with air circulation at 100 ± 5 °C for one hour. After cooling in a desiccator, the capsule was weighed and the drying repeated until constant weight. The result was expressed in g L^{-1} and obtained using the formula TDE = 50 x (a-b), where a = mass of the capsule with the extract and b = mass of the capsule (Instituto Adolfo Lutz, 2008). All analyses were performed in triplicate.

Determination of ash content

The ash content determination was carried out using the gravimetric method and is based on the elimination of volatile organic and

inorganic matter when the sample is incinerated at 550 °C ± 25 °C, without appreciable decomposition of the constituents of the mineral residue. It was pipetted 20 mL of sample into a porcelain crucible, previously tared, followed by evaporation of the sample with the aid of a heating plate to dryness and carbonization in a Bunsen burner. Afterwards, the crucible was inserted into a muffle at 550 °C ± 25 °C until the residue became white or gray, cooled in a desiccator and weighed, operation repeated until constant weight. The ashes were expressed in g L^{-1} and calculated using the formula Ashes = 50 x (a-b), where a = mass of the crucible with ashes and b = mass of the crucible (Instituto Adolfo Lutz, 2008). All analyses were performed in triplicate.

Determination of colour parameters

The colorimetric parameters using the L * a * b * and L * C * h ° colour spaces established by the CIE (Commission Internationale de l'Eclairage) were determined using the Delta Vista portable spectrophotometer (Delta Colour, Brazil), with illuminant D65 and observer at 10º, after calibration with white and black porcelain plate with light trap, coupled to the software i7 (Delta Colour). The samples were read in a disposable cuvette with 10 mm optical path and the results represent the average of nine readings, three readings for each sample of the experiment carried out in triplicate. Both colour spaces used allow the specification of colour perceptions in terms of a three-dimensional space. In the L * a * b * system, the axial L * is known as luminosity and extends from 0 (black) to 100 (white), the a * coordinate quantifies the colour variation from green (negative values) to red (positive values) and the coordinate b * the variation from blue (negative values) to yellow (positive values), with coordinates a * and b * varying from +60 to -60. The L * C * h ° system uses the same diagram as the L * a * b * colour space, but uses cylindrical coordinates instead of rectangular coordinates, being preferred by some industry professionals because this system correlates better with the shape how the human eye perceives colour. In the L * C * h ° system, the axial L * also indicates luminosity, C * represents saturation, and h is the hue angle. The saturation value C *,

represents the distance from the luminosity axis (L *) and starts at zero in the center. The hue angle starts on the + a * axis and moves counterclockwise. It is expressed in degrees (for example, 0° is red, 90° is yellow, 180° is green, 270° is blue e 360° is red).

The colorimetric parameters were measured after the maceration operation (time zero) and after 18 months kept in na amber glass container at room temperature.

Statistical analysis

For data analysis, it was used the Excel 2016 and the technique of descriptive statistics was applied using one-way analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$. Differences between means were tested with the Tukey-Kramer test (p-value < 0.05).

RESULTS AND DISCUSSIONS

The vinegars are classified and named according to the raw material used in their production, such as alcohol vinegar, fruit, cereal, vegetable, mixed vegetables, honey, compost (BRASIL 2012) and wine vinegar (BRASIL 2018). Vinegars obtained from fruits have a more complex composition than alcohol vinegar because it contains practically all the soluble substances that exist in the raw material, or that were formed in the alcoholic and acetic fermentation processes. The alcoholic solutions used for the manufacture of alcohol vinegar have a much more restricted composition, being basically formed by ethanol which undergoes the action of acetic bacteria being transformed into acetic acid. Alcohol vinegar when compared to fruit vinegar has economic advantages, since ethyl alcohol is much cheaper than fermented fruit, both of which are used as raw materials in the vinegar production processes, respectively.

Despite the economic advantages of alcohol vinegar, fruit vinegars are increasingly popular around the world due to their functional and sensory characteristics. These can be produced from different cultivars and also from their residues, using classic and modern techniques. As examples, it can be mentioned, kiwi vinegar (Bortolini et al. 2001), pineapple peel vinegar (Sossou et al. 2009; Raji

et al. 2012), blueberry vinegar (Su & Chien et al., 2010), ginger vinegar (Suman 2012), strawberry vinegar (Ubeda et al. 2013) persimmon vinegar (Ubeda et al. 2011). Zilioli (2011) studied the content of compounds of sensory importance and the identity patterns of vinegars produced from rice (*Oryza sativa*), sugar cane (*Saccharum officinarum*), corn (*Zea mays*), honey from *Apis melifera*, carambola (*Averrhoa carambola*), kiwi (*Actinia chinensis*), orange (*Citrus sinensis*), apple (*Malus domestica*), passion fruit (*Passiflora edulis*), grapefruit (*Citrus paradisi*) and grape (*Vitis* sp.). As well as their raw materials, these vinegars have a wide variety of organic acids, vitamins, minerals, phenolic compounds and several other compounds with important physiological functions (Solieri & Giudici, 2009).

The acai, an Amazon native fruit with nutritional and functional characteristics proven by the various works published in the literature, was macerated in alcohol vinegar produced by the company Virrosas (Manaus), in order to transform the alcohol vinegar in a product rich in functional compounds through a very simple physical operation. Figure 2 shows the TDE results obtained for the alcohol vinegars macerated with acai using diferente proportions and times.

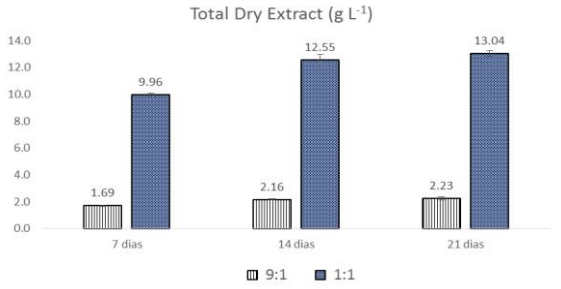


Figure 2. Determination of TDE in alcohol vinegars macerated with acai at different proportions and times

As can be easily seen in Figure 2, the marked difference observed in the TDE content was related to the proportion of alcohol vinegar: acai used, with higher values for the 1: 1 ratio when compared to 9: 1. The maceration time also caused an increase in the TDE content, however

the greatest difference observed was between the maceration times of 7 days and 14 days in the proportion 1: 1.

The determination of ash content aims to determine the minerals contained in the product and the values obtained for alcohol vinegars macerated with acai are shown in Figure 3.

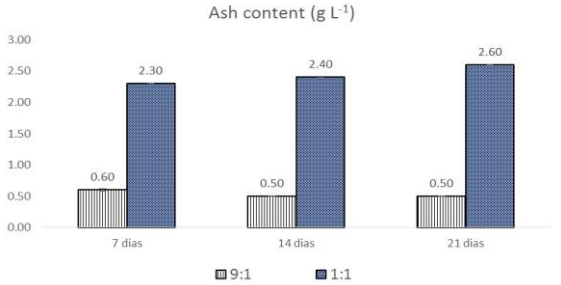
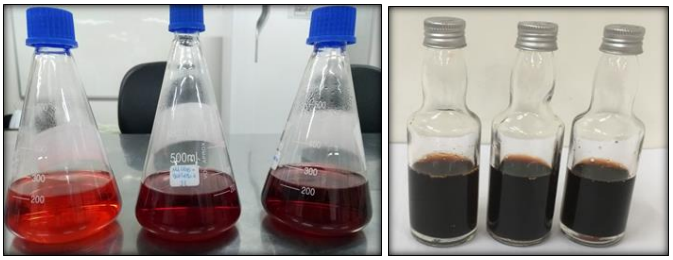


Figure 3. Determination of the ash content in alcohol vinegars macerated with acai at different times and proportions

For the ash content results obtained, a marked difference was observed between the vinegars macerated with the proportions of acai of 1: 1 and 9: 1, but similar values were obtained for the different maceration times. The considerations for the ash content are analogous to that found for the total dry extract of the vinegars, a marked difference was observed in the different proportions of alcohol vinegar: acai used (9: 1 and 1: 1) and with the trend of similar results for the different maceration times employed (7, 14 and 21 days) in the 9:1 ratio and a small increase in the 1: 1 ratio. The Brazilian legislation establishes for ash content a minimum value of 1 g L^{-1} and a maximum of 5 g L^{-1} for fruit vinegar and do not establish a limit for alcohol vinegar (BRASIL 2012). A fruit vinegar diluted and partially reconstituted with acetic acid has low values for ash content and very high values may indicate the addition of non-volatile substances. The alcohol vinegar macerated with acai in the proportion 1: 1 and at all times, employed presented a value in accordance with the legislation for fruit vinegar.

In Figure 4, the colouring of alcohol vinegars macerated with acai at zero time and after 18 months stored at room temperature and in an amber glass container can be seen.



AFTER 18 MONTHS STORAGE

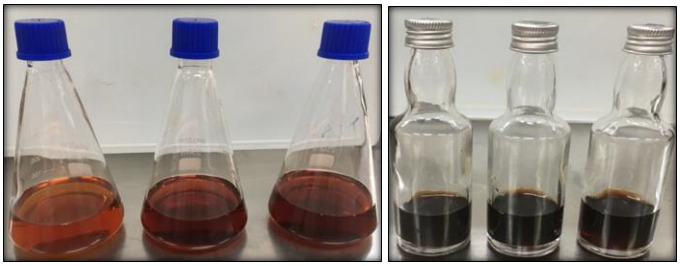


Figure 4. Colour of vinegars macerated with acai for 7, 14 and 21 days in the proportions 9: 1 (Erlenmeyer) and 1: 1 (bottle) at time zero and after 18 months stored at room temperature and in amber glass container.

Through visual observation (Figure 4) it can be easily noticed the difference in colour of the alcohol vinegar after the maceration with acai for both proportions (9:1 and 1:1) used. Also, it is noticeable the changes in colour between time zero and after the storage for 18 months for the 9: 1 ratio (Erlenmeyer), whereas for the 1: 1 ratio (bottles) it is not possible to visually verify changes in the colouring between time zero and after the storage for 18 months.

In Table 1, are shown the results obtained in the determination of colour parameters (L*a*b* C*h°) of all alcohol vinegars macerated with acai visualized in Figure 4. For all parameters measured there were statistically differences between the proportions of alcohol vinegar: acai used (9: 1 and 1: 1).

For the measurements of colour parameters after the experiments, it was observed for the proportion 9: 1 (alcohol vinegar: acai) that the values of L^* were not statistically different between the three maceration times (7, 14 and 21 days) employed. For the others parameters, a^*, b^*, C^* and h°, the values obtained for 14 and 21 days were statistically higher than the value found for 7 days. These

results can be easily visualized in Figure 4, where it is perfectly visible that there is a difference in colour between 7 days and the other two times (14 and 21 days) and that there is no visible difference in colour between 14 and 21 days of maceration. However, for the proportion 1: 1 (alcohol vinegar: acai), although it is difficult to see differences between the three maceration times (Figure 4), significant differences were observed for the colour parameters measured in relation to the maceration time. For the parameters a^*, b^* and C^* the values obtained in the maceration for 7 days were statistically higher than 14 and 21 days, already for the h° the values obtained when the maceration occurred during 14 and 21 days were statistically higher when compared to 7 days. The results obtained for both proportions (9: 1 and 1: 1) employed, demonstrated that there were no noticeable (Figure 4) and significant (Table 1) differences in colour parameters between 14 and 21 days and therefore a maceration time with acai beyond 14 days did not promote any benefits for the colouring of the alcohol vinegar.

For the measurements of colour parameters after 18 months storage a different pattern was observed when compared to measurements performed after the experiments. For the proportion 9: 1 (alcohol vinegar: acai) the values obtained for coordinate a^* followed the same trend observed in the previous measurement and the values obtained for 14 and 21 days maceration were significantly higher than for 7 days. For the other parameters, L*, b*, C* and h° an opposite trend occurred and the values obtained for 7 days were significantly higher when compared to 14 and 21 days, with the exception that for only the C* results it was not observed statistically difference between the three maceration times used. For the proportion 1:1 (alcohol vinegar: acai) the only statistical difference observed was for the coordinate b^*, which followed the same trend of the previous measurement, with higher values for 7 days when compared to 14 and 21 days.

When comparing the results obtained after the experiment (time zero) and after 18 months of storage, for each of the measured colour parameters and for each sample, it can be observed that the marked differences occurred in the 9: 1 ratio (Table 1), which can also

be easily visualized in Figure 4. For the 1: 1 ratio, despite the statistical differences found, the differences were not so marked and the colour difference is not visible.

Table 1. Measurement of colour parameters of the alcohol vinegars macerated with acai at different times and proportions at time zero and after 18 months storage.

	Measure after experiment						Measure after 18 month				
	L*	**a***	**b***	**C***	**h°**		**L***	**a***	**b***	**C***	**h°**
Day	**alcohol vinegar: acai (9: 1)**					**Day**	**alcohol vinegar:acai (9:1)**				
7	15.37 ± 1.20^{Aa}	21.26 ± 2.08^{Ba}	10.13 ± 1.78^{Bb}	23.56 ± 2.63^{Ba}	25.29 ± 1.92^{Bb}	**7**	16.12 ± 1.58^{Aa}	13.94 ± 1.61^{Ab}	20.11 ± 0.45^{Aa}	24.50 ± 1.19^{Aa}	55.38 ± 2.78^{Aa}
14	15.08 ± 0.95^{Aa}	28.58 ± 0.62^{Aa}	19.46 ± 1.24^{Aa}	34.57 ± 1.20^{Aa}	34.21 ± 1.14^{Aa}	**14**	7.84 ± 0.94^{Bb}	19.68 ± 0.21^{Bb}	11.49 ± 1.58^{Bb}	22.83 ± 0.75^{Ab}	30.18 ± 3.53^{Ba}
21	15.12 ±1.70^{Aa}	28.70 ± 0.81^{Aa}	19.26 ± 1.78^{Aa}	34.58 ± 1.64^{Aa}	33.80 ± 1.80^{Aa}	**21**	7.71 ± 1.75^{Bb}	18.97 ± 0.15^{Bb}	11.22 ± 2.69^{Bb}	22.13 ± 1.53^{Ab}	30.26 ± 5.66^{Ba}
Day	**alcohol vinegar: acai (1: 1)**					**Day**	**alcohol vinegar: acai (1: 1)**				
7	2.50 ± 0.13^{Ba}	3.59 ± 0.37^{Aa}	0.67 ± 0.15^{Aa}	3.66 ± 0.38^{Aa}	10.59 ± 2.12^{Bb}	**7**	1.57 ± 0.12^{Ab}	1.42 ± 0.32^{Ab}	0.52 ± 0.08^{Aa}	1.53 ± 0.27^{Ab}	21.33 ± 7.37^{Aa}
14	4.77 ± 0.05^{Aa}	1.41 ± 0.37^{Ba}	-0.17± 0.14^{Bb}	1.42 ± 0.37^{Ba}	349.83 ± 3.54^{Aa}	**14**	1.46 ± 0.27^{Ab}	1.20 ± 0.52^{Aa}	0.32 ± 0.11^{Ba}	1.24 ± 0.53^{Aa}	15.84 ± 5.15^{Ab}
21	1.89 ± 0.23^{Ca}	1.47 ± 0.23^{Ba}	-0.42 ± 0.18^{Cb}	1.53 ± 0.25^{Ba}	344.12 ± 5.97^{Aa}	**21**	1.57 ± 0.21^{Aa}	1.64 ± 0.37^{Aa}	0.39 ± 0.11^{Ba}	1.68 ± 0.37^{Aa}	13.50 ± 2.53^{Ab}

Different capital letters in the same row for each proportion alcohol vinegar: acai indicate statistically significant difference at $p < 0.05$ according to Tukey-Kramer Test
Different lower letters for each sample after experiment and after 18 months indicate statistically significant difference at $p < 0.05$ according to Tukey-Kramer Test

In general terms, there were marked differences in colorimetric parameters between both proportions of alcohol vinegar: acai used (9:1 and 1:1). However, when analysing the maceration time, the marked difference was found between 7 days and the other times used (14 and 21 days), with the values found for all parameters analysed being similar between 14 days and 21 days. The maceration time interfered with the results obtained and for almost all analyses performed and no marked differences were observed between 14 days and 21 days of maceration, indicating that the maceration should be carried out for a maximum of 14 days, which probably must be related to the principles of maceration process. The maceration process does not lead to the exhaustion of the active compounds due to the saturation of the extraction liquid and diffusional balance between the extraction medium and the interior of the plant cell, in this case the acai berry.

Bastante *et al.* (2010) studied the maceration of sherry vinegar with orange peel for 3, 7 and 14 days and reported that the

longer maceration time (14 days) did not significantly increase the antioxidant activity. After establishing the maceration time, the maceration of different fruit peels (orange, lemon, grapefruit and lime) were evaluated and the results demonstrated that the macerated vinegars showed greater antioxidant activity due to the presence of phenolic compounds such as glycosylated flavanones (hesperidin, neohesperidine and naringin). The authors demonstrated that several phenolic compounds were extracted from the fruit skins during the vinegar maceration process. In this study, certainly the same can be said for the maceration of acai in alcohol vinegar, and different kinds of compounds were transferred from the acai to the alcohol vinegar as observed in the results obtained for the TDE (Figure 3), ashes content (Figure 4) and colour (Figures 1 and 4, Table 1).

Anthocyanins are blue, red or purple pigments commonly found in fruits of many plants. The coloured pigments of anthocyanin from berries, blackcurrants, and other types of red to blue-coloured fruits are strong antioxidants. Anthocyanins possess antidiabetic, anticancer, anti-inflammatory, antimicrobial, and anti-obesity effects, as well as prevention of cardiovascular diseases (Hock et al. 2017). According to Pacheco-Palencia et al. (2008), the anthocyanin profile of acai was characterized by the predominance of cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside and peonidin-3-rutinoside, the presence of the las two related to acai species. Regarding the phenolic compounds profile, many other substances have been identified in acai, e.g. ferulic acid, p-hydroxybenzoic, gallic, protocatechuic, ellagic, vanillic, p-coumaric, caffeic, benzoic, syringic, chlorogenic acids, ellagic acid glucoside and resveratrol (Del Pozo-Insfran et al. 2004; Gordon et al. 2012). In addition, Chin et al. (2008) reported another 22 compounds including nine lignans, four simple benzenoids, three flavonoids, a benzoquinone, three monoterpenoids and two norisoprenoids.

The studied process, maceration of acai in alcohol vinegar, can be easily used by any vinegar manufacturer and allows the diversification of products, the value adding to the acai from the extractivism in the state of Amazonas and the elaboration of a product

with a long shelf life from a fruit highly perishable. Future work will be carried out to identify and quantify the compounds present in alcohol vinegar macerated with acai.

CONCLUSION

Considering the experimental conditions employed and the results obtained for the determination of TDE, ash content and colour parameters, it is concluded that there was a transfer of substances present in the acai to the alcohol vinegar. The differences were marked between the proportion of alcohol vinegar: acai used, with significantly higher differences for the 1: 1 ratio when compared to 9: 1. Regarding the maceration time, the results indicating that the maceration should be carried out for a maximum of 14 days.

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