

Production and characterization of acetic fermentation with different fruit peels

Produção e caracterização da fermentação acética com diferentes cascas de frutas

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Abstract

Acetic fermentation or vinegar is the product obtained from fermented alcoholic wort, cereals or hydroalcoholic mixture with a minimum volatile acidity of 4g/100m.L⁻¹. The present study produced, characterized and correlated the total dry extract with the density of the acetic fermented produced from the pineapple and apple peels. The fermentation process used pineapple and apple peel with culture medium, 10% hydroalcoholic solution, room temperature between 28 ± 2°C and irrigation 4 (four) times a day, without aeration for 12 days. Physico-chemical determinations of volatile acidity, density, dry extract, pH and alcohol content at 20°C were performed. The ANOVA variance analysis and the 5% Tukey test identified a difference between the analyzed acetic fermented. The acetic fermentations analyzed showed results within the established by Brazilian legislation. The relationship between the physical-chemical variables and the acetic fermented was studied by Principal Component Analysis (PCA). It was obtained that, with 5 components, more than 97.9% of the variability of the data were explained. There was a significant correlation between dry extract and vinegar density. The acetic fermented produced have similar physical-chemical characteristics to those produced industrially, which makes them suitable for consumption as commercial vinegars.

Keywords: Family farming; Fruit; Reuse; Agro-industrial waste.

Resumo

A fermentação acética ou vinagre é o produto obtido a partir de mosto alcoólico fermentado, cereais ou mistura hidroalcoólica com acidez volátil mínima de 4g/100m.L⁻¹. O presente estudo produziu, caracterizou e correlacionou o extrato seco total com a densidade do fermentado acético produzido a partir das cascas de abacaxi e maçã. O processo de fermentação utilizou casca de abacaxi e maçã com meio de cultura, solução hidroalcoólica a 10%, temperatura ambiente entre 28 ± 2°C e irrigação 4 (quatro) vezes ao dia, sem aeração por 12 dias. Foram realizadas determinações físico-químicas de acidez volátil, densidade, extrato seco, pH e teor de álcool a 20°C. A análise de variância ANOVA e o teste de Tukey a 5% identificaram diferença entre os fermentados acéticos analisados. As fermentações acéticas analisadas apresentaram resultados dentro do estabelecido pela legislação brasileira. A relação entre as variáveis físico-químicas e o fermentado acético foi estudada por Análise de Componentes Principais (PCA). Obteve-se que, com 5 componentes, mais de 97,9% da variabilidade dos dados foram explicados. Houve uma correlação significativa entre o extrato seco e a densidade do vinagre. Os fermentados acéticos produzidos possuem características físico-químicas semelhantes aos produzidos industrialmente, o que os torna adequados para consumo como vinagres comerciais.

Palavras-chave: Agricultura familiar; Fruta; Reuso; Resíduos agroindustriais.

Resumen

La fermentación acética o vinagre es el producto obtenido a partir de mosto alcohólico fermentado, cereales o mezcla hidroalcohólica con una acidez volátil mínima de $4\text{g}/100\text{m.L}^{-1}$. El presente estudio produjo, caracterizó y correlacionó el extracto seco total con la densidad del producto acético fermentado producido a partir de cáscaras de piña y manzana. El proceso de fermentación utilizó cáscara de piña y manzana con medio de cultivo, solución hidroalcohólica al 10%, temperatura ambiente entre $28 \pm 2^\circ\text{C}$ y riego 4 (cuatro) veces al día, sin aireación durante 12 días. Se realizaron determinaciones fisicoquímicas de acidez volátil, densidad, extracto seco, pH y contenido de alcohol a 20°C . El análisis de varianza ANOVA y la prueba de Tukey al 5% identificaron diferencias entre los fermentados acéticos analizados. Las fermentaciones acéticas analizadas presentaron resultados dentro de lo establecido por la legislación brasileña. La relación entre las variables fisicoquímicas y la fermentación acética se estudió mediante el Análisis de Componentes Principales (PCA). Se encontró que, con 5 componentes, se explicaba más del 97,9% de la variabilidad de los datos. Hubo una correlación significativa entre el extracto seco y la densidad del vinagre. Los productos fermentados acéticos producidos tienen características fisicoquímicas similares a los producidos industrialmente, lo que los hace aptos para su consumo como vinagres comerciales.

Palabras clave: Agricultura familiar; Fruta; Reutilizar; Residuos agroindustriales.

1. Introduction

Fermentation can be defined in a general sense as any transformation process produced from microorganisms that occurs in the presence or absence of air (Jones *et al.*, 2019, Viana *et al.*, 2017). Fermentation is the biochemical process in which microorganisms remove the nutrient material they need from the medium in which they live, producing substances, under the catalytic action of enzymes, which the industry uses (Viana *et al.*, 2017, Evangelista, 2008). Fermentation produces a variety of products, such as food, beverages, organic acids, solvents, esters, amino acids, enzymes, polysaccharides, vitamins, antibiotics and hormones (Cunha, 2010). Among the fermentative processes, alcoholic, acetic and lactic fermentation stand out. These processes break the substrate into pyruvate and convert it into another product, such as ethanol, acetic acid, lactic acid. Acetic fermentation consists of converting ethyl alcohol into ethanoic acid (acetic acid) by means of acetic bacteria.

Acetic bacteria belong to a class of microorganisms of broad economic interest, both because of the production of ethanoic acid and because of changes in food and drink. (Hoffmann, 2006). These organisms have excellent adaptation to sugary environments. Its ability to convert ethyl alcohol into ethanoic acid is one of the characteristics that popularized its use in the production of vinegars (Bartowysky & Henschk, 2008). Acetic fermented or vinegar is the product obtained from the acetic fermentation of the alcoholic fermented fruit must, cereals, sugary products, vegetables, honey, vegetable mixture or hydroalcoholic mixture with a minimum volatile acidity of $4\text{g}/100\text{m.L}^{-1}$, expressed in acetic acid. The acetic fermented can have several classifications, according to the origin of the raw material, being called acetic fermented or vinegars, followed by the name of the raw material of origin. They can present optional ingredients such as herbs, spices, salt, among others, in sufficient quantities to impart a peculiar aroma and flavor (Brasil, 2012).

Brazilian vinegars are produced from ethyl alcohol, constituting a 4% acetic acid solution, this acid concentration being the minimum required by law, with the addition of an antioxidant that acts as a stabilizer (Netto, 2006). In Brazilian territory, the manufacture of vinegar with dilution of ethanoic acid of non-fermentative origin is prohibited. For concentrated vinegars, the same characteristics will apply, respecting the proportions of concentration (Brasil, 2012, Aquarone *et al.*, 2001). Vinegar can be produced by consecutive processes of alcoholic and acetic fermentation. In the first, the carbohydrate is converted into ethyl alcohol and in the second, fermentative oxidation occurs which turns ethyl alcohol into ethanoic acid. (Veloso, 2013, Schmoeller, 2011). Deformed or non-standard fruits are discarded after harvest. In this way, these fruits can be used to manufacture vinegar. (Ubeda *et al.*, 2011, Evangelista, 2008, Bortolini, Sant'anna & Torres 2001).

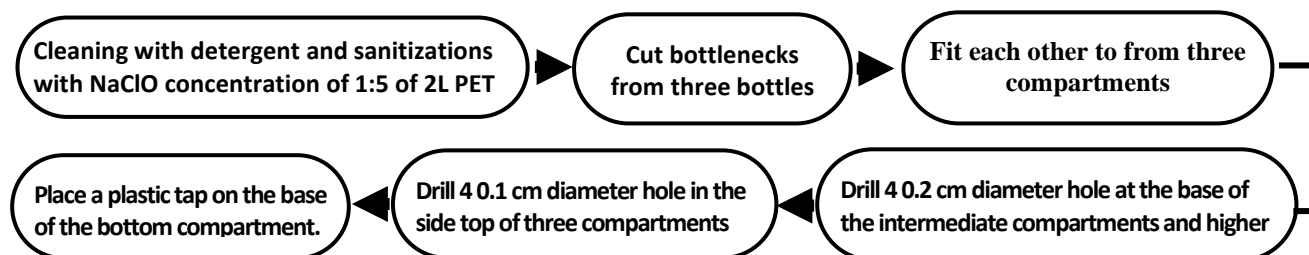
Fruit peels represent a good option for the production of vinegar, as they have a large amount of soluble solids and nutrients (Paganini *et al.*, 2003). The manufacture of vinegar using fruit peels represents an alternative to add commercial value to deformed, injured or advanced ripening fruits. (Abud, Silva & Araújo, 2012, Tessaro *et al.*, 2010, Santos *et al.*, 2008).

Thus, this research aimed to produce, characterize and correlate the total dry extract with the densities of the acetic fermented obtained from the pineapple and apple peels by slow spontaneous fermentation.

2. Methodology

During the months of April and May of the year 2020, PET bottles were purchased in snack bars and pizzerias in the Municipality of Paraíso do Tocantins. The bottles were used in the construction of a reactor (Figures 1 and 2) for the production of acetic fermented pineapple and apple peels. The reactor was developed according to a flow chart (Figure 1), applying the method used by Santos *et al.* (2008).

Figure 1. Flowchart of the reactor construction (PET bottle) for acetic fermentation of fruit peels.



Source: Santos et.al. (2008).

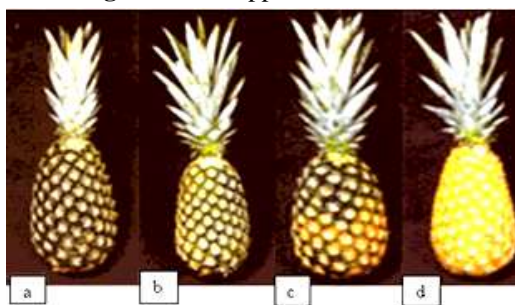
Figure 2. (A) - Sanitization of PET bottles. (B) - Removal of the neck of the PET bottle. (C) - Holes at the base of the intermediate and upper compartments and (D) - Fit the bottles together to form the reactor.



Source: Authors (2021).

The experiment was carried out at the processing and vegetable unit of the Federal Institute of Education of Tocantins - IFTO: *Campus* Paraíso. Pineapples (*Ananas comosus*) and gala apples (*Red delicious*), used for the production of acetic fermented, were purchased in supermarkets and fruit stores in the Municipality of Paraíso do Tocantins. Pineapples were purchased according to the maturation stages "B" and "C" in Figure 3 and apples according to the classification "C" and "D" in Figure 4.

Figure 3. Pineapple classification.



Source: <https://www.monografia.com/pt/trabalhos2/processed-foods/processed-foods2.shtml>

Figure 4. Apple classification.



Source: Betinelli (2017).

The fermentation process was developed using 2 liters of 10% hydroalcoholic solution (v/v), 2 kg of pineapple peel and 1 kg of apple peel. Fruit peels were used as a culture medium, as they are a source of microorganisms that transform alcohol into acetic acid. The irrigation system used was the introduction of the alcoholic solution at the top of the reactor and after 2 hours, the liquid was collected by the tap at the base of the reactor. This process was repeated 6 (six) times a day for 12 days at room temperature between 28 ± 2 °C without aeration (Santos et al., 2008). After the fermentation process, the acetic fermentates produced by the slow fermentation process were filtered, stored and sterilized in a glass container. The physicochemical determinations used for the determination of volatile acidity ($\text{g}\cdot\text{mL}^{-1}$), density at 20°C ($\text{g}\cdot\text{mL}^{-1}$), dry extract ($\text{g}\cdot\text{L}^{-1}$), hydrogen potential and alcohol content at 20°C ($\% \text{v}\cdot\text{v}^{-1}$) followed the methodological procedures of Instituto Adolfo Lutz (Instituto Adolfo Lutz, 2008).

The physicochemical, acidity, density, dry extract, hydrogen potential and alcohol content analyzes were processed in quadruplicate and their results submitted to analysis of variance. To verify whether there was a significant difference between the results, ANOVA and Tukey's test were applied with a significance level of 5% between the means of the response variables. Statistical correlation between total dry extract and relative density at 20 °C was performed using Microsoft Excel for Windows (2003). The Principal Component Analysis (PCA) analyzed the data in which the treatments are related by interrelated response variables, where the objective was to group the physical-chemical variables by similarity. Principal Component Analysis (PCA) was used to group physical and chemical variables according to similarity. PCA was performed on standardized data to avoid the effect of different magnitude levels of response variables. Data standardization for each response variable was performed by subtracting each value by its mean divided by the standard error. All statistical analyzes were performed using Sisvar version 5.6 (Ferreira, 2019). The ACP analysis was performed with the PAST software (Hommer, Harper & Ryan, 2001).

3. Results and Discussion

The results of the physical-chemical parameters analyzed for the acetic fermented produced with pineapple (FAC pineapple) and apple (FAC apple) peels compared to commercial red wine vinegar (VCVT) are shown in Table 1.

Table 1. Physical-chemical characteristics of artisanal fermented fermenters compared to commercial vinegar.

Vinegar	Acidity (g.mL ⁻¹)	Density 20° C (g.mL ⁻¹)	Dry extract (g.L ⁻¹)	Hydrogen potential pH	Alcohol content % (v/v)
VCVT	4.42 ^C ± 0,007	1.0125 ^A ± 0,000	10.59 ^A ± 0,006	2.74 ^B ± 0,006	0.59 ^A ± 0,006
FAC pineapple	4.53 ^B ± 0,004	1.0109 ^C ± 0,000	6.90 ^C ± 0,015	2.91 ^A ± 0,008	0.45 ^B ± 0,010
FAC apple	4.55 ^A ± 0,010	1.0120 ^B ± 0,000	8.80 ^B ± 0,005	2.71 ^C ± 0,005	0.28 ^C ± 0,006
C. V (%)	0.16	0.01	0.11	0.24	1.33
Legislation*	≥ 4,0 (g.mL⁻¹)	-----	≥ 6,0 (g.L⁻¹)	-----	≤ 1,0 (% v/v)

Equal letters in the columns mean that the types of acetic fermented did not differ from each other ($P > 0.05$) by the Tukey test. C.V (%) coefficient of variation.

* Normative Instruction N°. 6/2012 of MAPA

Source: Authors (2021).

According to the analysis in Table 1, it is observed that for all variables studied, acidity, density, total dry extract, pH and alcohol content indicate that the samples showed heterogeneity based on the Tukey test at 5% significance. There was a significant difference ($P \leq 0.05$) between the acetic fermented pineapple, apple and commercial red wine vinegar.

The acetic fermented produced with pineapple and apple peels from an artisanal process showed significant differences at the level of 5% in relation to Commercial Vinegar from the large-scale production industry, indicating the influence of the type of processing on the final quality of the product. The results obtained for the physical and chemical analyzes showed values in accordance with Normative Instruction N°. 6, of April 3, 2012 from the Ministry of Agriculture, Livestock and Supply (Brasil, 2012). The density and hydrogen potential do not have limits specified in the referred legislation.

Acidity influences the sensory acceptance of the product, since the percentage of acetic acid in the acetic fermented is directly proportional to the sensorially perceived acidity (Granada, Mendonça *et al.* 2000; Tesfaye *et al.*, 2002). Rizzon & Miele (1998) found average volatile acid values of 4.4% for red wine vinegar and 4.3% for white wine vinegar. Silva *et. al.* (2008), reusing fruit peels for the production of vinegar found acidity in the acetic fermented produced with the pineapple peels equal to 4.55 ± 0.15 (g.mL⁻¹) and 4.54 ± 0.08 (g.mL⁻¹) for apple. Moreira, and contributors (2017), developing acetic fermentation with pineapple peel, found acidity of 1.3 g.mL⁻¹. Violi, and contributors (2021), producing artisanal fermented acetic acid with apple peel found a value of 4.56 ± 0.01 g.mL⁻¹ for volatile acidity.

The relative density at 20 ° C is based on the relationship between the specific weight of the sample at 20 ° C in relation to the specific weight of water at 20 ° C. As for density, Rizzon & Miele (1998) found lower values from the analytical characterization of commercial acetic fermented wines from Brazil, with 1.0103 g mL⁻¹ being acetic fermented red wines and 1,0009 g.mL⁻¹ of wine white. Moreira, and contributors (2017), found a density value of 1009.9 g/L in acetic fermented produced with pineapple peel. Violi *et. al.* (2021) found a density value of 1.0108 ± 0.0200 g.mL⁻¹ in acetic fermentation with apple.

The determination of the dry extract of acetic fermented is an attempt to avoid fraud that has been widely used in the past, since very low or very high levels of dry extract may indicate adulteration of the product (Takemoto, 2000). The dry extract value for the acetic fermented pineapple found in this work was lower than that reported by Diniz (2017) who found for the total dry extract of the fermented pineapple peel 44.02 g.L⁻¹. Marques, and contributors (2010), evaluating the identity and

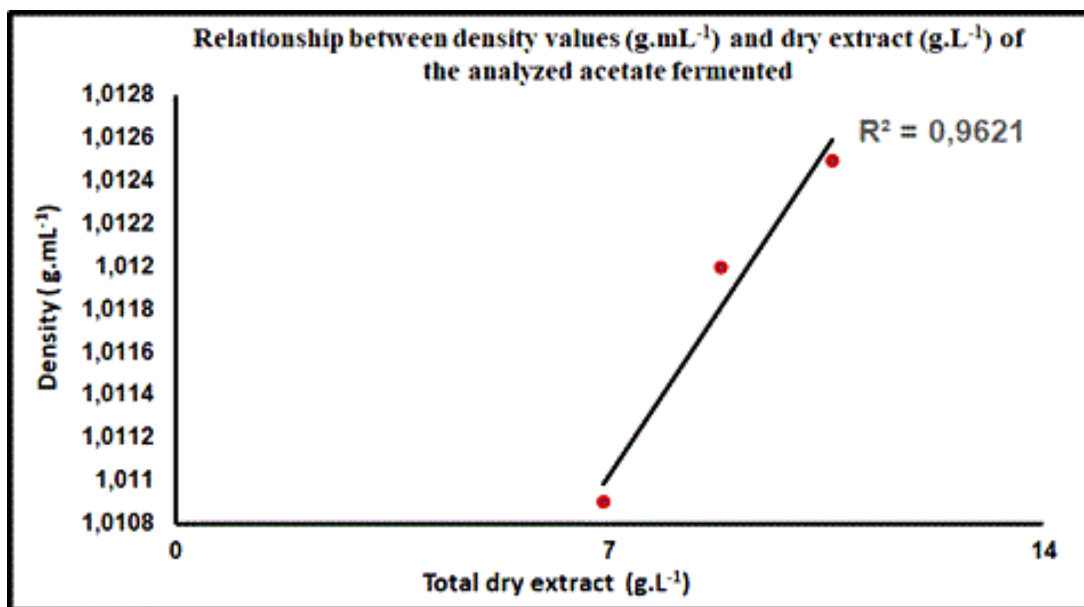
quality standards of commercial acetic fermented fruits and vegetables found values for the total dry extract of the fermented apple equal to $8.9 \pm 0.311 \text{ g.L}^{-1}$, which is also very close to the value of this study. Rizzon and Miele (1998), studying the analytical characteristics of commercial acetic fermented produced with red wine, found values for the dry extract equal to 9.78 g.L^{-1} , a value lower than that found in this study.

Pedroso (2003) evaluating the acetic fermented apple found a variation of 3.10 ± 0.26 for the pH. Marques *et al.* (2010) found variations in pH values for the acetic fermented red wine equal to 2.69 ± 0.045 and 3.10 ± 0.017 for the acetic fermented apple. Santos *et al.* (2008), found pH variations equal to 2.72 ± 0.04 for the acetic fermented produced with apple and 2.78 ± 0.13 for the acetic fermented with the pineapple peels. The pH found by Santo et al is higher than the pH found in this study. Moreira, and contributors (2017) found a pH value equal to 3, 9 for the acetic fermented produced with pineapple peel.

Viroli *et al.* (2021) found alcohol content of acetic fermented produced with apple equal to 0.21 ± 0.01 (% v.v⁻¹). Santos *et al.* (2008) found variations in the alcohol content for the acetic fermentation of pineapple and apple equal to 0.25 ± 0.08 (% v.v⁻¹) and 0.43 ± 0.12 (% v.v⁻¹), respectively. Marques, *et al.* (2010), analyzing the alcoholic content in acetic fermented apple found a value of 0% in the analyzed samples. Possibly, the methodology used by the authors to determine the alcohol content was not sensitive enough to detect the low concentrations of alcohol in the acetic fermented.

According to White (1971) and Marques *et al.* (2010), an efficient conversion occurs with the transformation of 70% of alcohol into acid, reaching an efficiency of 90 to 98%. The statistical correlation between the values of dry extract and density resulted in a correlation coefficient R^2 , which represents the existence of a significant correlation ($P \leq 0.01$) between the two physical and chemical parameters analyzed. The dry extract and the density of the acetic fermented are directly proportional. The graph below (Figure 05) shows a linear regression between the two determinations, with a coefficient (R^2) of 0.9621, corroborating the existence of linearity between the dry extract content and sample density.

Figure 5. Relationship between values of density relative to 20°C (g.mL^{-1}) and dry extract (g.L^{-1}) of the analyzed acetate fermented.



Source: Authors (2021).

Marques (2008), researching on physical-chemical, nutritional and sensory characteristics of vinegars of different raw materials, found a value for linear relationship (R^2) between the density values at 20 °C and the extract equal to 0.8580. Lira

(2004) states that a linear correlation is classified as very strong when the modules of the values of the correlation coefficient are between 0.90 and 1.0. Thus, according to the author's classification, the result of the R^2 found by Marques showed a strong correlation, while the R^2 found for the correlation between density and dry extract is classified with a lot strong.

The Table 2 describes the weights of the main components CP1 and CP2 for all variables studied.

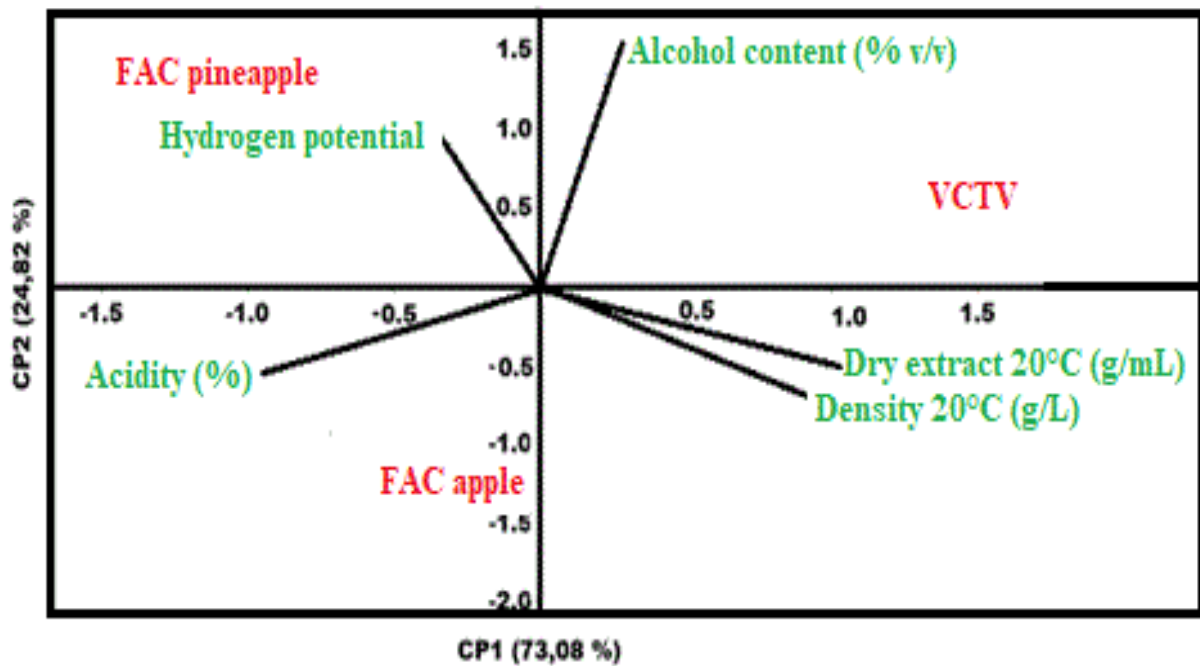
Table 2. Description of the main components CP1 and CP2 for the physical and chemical variables analyzed.

Variable	PC1	PC2
Acidity % (p/v)	-0.50441	-0.22557
Density 20° C (g/mL)	0.50059	-0.11412
Dry extract (g/L)	0.51746	-0.053882
Hydrogen potential pH	-0.40305	0.5658
Alcohol content % (v/v)	0.25451	0.78298

Source: Authors (2021).

Principal Component Analysis (PCA) provided a simplified interpretation (Figure 6) of the relationships between the response variables as well as the samples, facilitating the visualization of the response variables and correlating them. The main components CP1 and CP2 (orthogonal axes) explained 97.79% of the total data variability (Table 03), 73.08% being explained by the first component and 24.82% by the second component. The sum of main components I and II ($\geq 75\%$) adequately presented the variability between the samples (Abdi & Williams, 2010).

Figure 6. Analysis of main components of acetic fermented handcrafted compared to commercial vinegar.



VCTV- commercial red wine vinegar.
 FAC-pineapple-fermented acetic pineapple peel
 FAC apple - fermented acetic apple peel.
 Source: Authors (2021).

Table 3. Principal components (CP), eigenvalues (Eigenvalue) and percentage of explained variance.

PC	Eigenvalue	% Variance
1	3.65707	73.08
2	1.24204	24.82
3	0.0974318	1.947
4	0.0053429	0.10677
5	0.00228256	0.045613

Source: Authors (2021).

According to Figure 6, the treatments VCTV, FAC pineapple and FAC apple are respectively in the upper right, upper left and lower left areas of the graph. According to Table 3 and Figure 6, in the first main component CP1, the variables acidity, density and dry extract stood out and for the second main component CP2 the alcohol content and pH. There is a high correlation between the variables density and dry extract. This high correlation was verified by forming acute angles between them, a vector of greater length and proximity to the CP1 axis. The VCVT Treatment was related to the highest values of alcohol content, density and dry extract and lower acidity. The FAC pineapple treatment was related to the highest pH and the FAC apple treatment to the highest acidity.

4. Conclusion

The acetic fermentation production process showed ease of assembly, production and a small amount of input for the production of vinegar. The acetic fermentation produced with fruit peels in a reactor produced with PET bottles, showed physical-chemical characteristics similar to the fermented ones produced industrially, which made them apt to be commercialized and consumed as vinegars.

It is suggested that new studies on the production of vinegar with fruit peels be carried out to promote the generation of family income and local economic development.

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