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Mango Peel as a Substrate for the Production of Citric Acid

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ABSTRACT

Citric acid production is important at an industrial level because flavored most of the food products. In the present century, the re-use of agro-industrial waste products has become very important, nonetheless in Mexico, a high amount of agricultural residues is discarded, among which are non-marketed such as mango fruits. The mango fruit is an important income for families that sustain themselves from this crop and contains viable amounts of fiber, carbohydrates, ash, and carotenes, among others. Thus the objective of this work was to evaluate the production of citric acid using the mango peel of six commercial varieties from southern Chiapas, as a substrate in fermentation, using Penicillium spp. During fermentation, a reduction in the pH value was observed in all treatments below 5.0 and Brix degrees less than 2.0. Two points of maximum acidity were observed in the growing medium, on day 3 with the manila, criollo, and tomy varieties, and on day 6, the rest of the treatments. The maximum concentration of soluble protein was found with the ataúlfo variety and the maximum concentration of citric acid obtained was found with the ataúlfo variety. The mango fruit peel could be a substrate with potential use for citric acid production.

Keywords: *Acidity, Fruit, Brix, pH, Citric acid*

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INTRODUCTION

Citric acid, a compound commonly used in foods and beverages, has become one of the most important organic acids for providing freshness for long periods. This compound meets the requirements of being a non-toxic preservative and acidulant (Juvín Vallejo, 2021), only in 2021, its consumption in Mexico reached 95 thousand tons.

Agro-industrial needs have placed citric acid as an essential component in the food sector, due to its pleasant flavor and the property of exalting aromas. Therefore and because of the current use of sustainable biological alternatives, this compound is generated from different agro-industrial residues (Magalhaes *et al*., 2023).

SIAP (2020) reports indicate that 40% of the annual production of the mango crop becomes waste in Mexico, and is difficult to consider it as a raw material for biological processes, due to the lack of technological development for its industrialization (Valdés *et al*., 2020).

A strategy that is being developed in the 21st century, to prevent them from ending up in garbage dumps, landfills, or being incinerated, is reuse, which implies giving it a second use, taking advantage of its biochemical and physicochemical properties to obtain other beneficial products. for food or cosmetic application (Cuadrado-Osorio *et al*., 2022; Rodríguez-Martínez *et al*., 2022; Magalhaes *et al*., 2023).

Agricultural waste from mango cultivation in Mexico has the potential to be used in transformation processes and generate viable products with the potential for use in food and cosmetics (Liu *et al*., 2023), as they contain viable amounts of fiber, carbohydrates, ashes, carotenes, among others (Corredor & Pérez., 2018; Sorrenti *et al*., 2019).

Based on the above, the objective of this work is the use of mango peel flour from six commercial varieties from southern Chiapas as a substrate to obtain citric acid by fungal fermentation with *Penicillium spp*.

MATERIALS AND METHODS

The raw material was obtained from the local markets of the Soconusco region, Chiapas. The varieties were distributed in the municipalities of Tapachula, Mazatan, Huehuetan, Villa Comaltitlan, Cacahoatan, and Tuxtla Chico.

The preparation of the flour for each variety of mango was carried out by obtaining the fruit, washing it with running water and pante soap (zote®), we use a mango peeler, and after that, we cut it into small slices to facilitate the drying process, placing on flat trays, dry the sample in the sun for three days.

After drying, it was processed in a blender and passed through sieves (350, 700, and 1300 mesh) to obtain fine flour.

The analysis of the peel of each mango variety was carried out before drying, pH, ashes, titratable acidity, brix degrees, and humidity were measured.

The experimental design consisted of seven treatments, based on two conditions: 1) without mango peel flour (control treatment) and 2) with mango flour (other treatments). The control treatment was prepared with the base mineral medium for the growth of *Penicillium*.

The composition of the mineral base medium was: 15 g of glucose, 1 g of ammonium nitrate, 0.5 g of monopotassium phosphate, 0.5 of hepta-hydrated magnesium sulfate, 0.1 g of hepta-hydrated ferrous sulfate, for 1 liter of medium.

The treatments that include mango peel flour were established based on the number of brix degrees obtained in the analysis of the peel and the relationship that 1 brix degree corresponds to 1 g of sucrose in 100 g of solution. They were established in 250 mL Erlemeyer flasks with a working volume of 100 mL. Considering the values of brix degrees per variety of mango fruit, 10% of the sugar source was replaced by amounts of flour added to the mineral base medium, in triplicate / mango variety. The *Penicillium sp* strain was supplied by the strain collection of "Instituto de Biociencias" from "Universidad Autónoma de Chiapas". Test tubes were inoculated with 10 mL of formulated medium for each variety of mango, allowing it to incubate for 3 days. Subsequently, Erlenmeyer flasks were inoculated. Physicochemical tests were performed on days 0, 3, 6, and 9 of growth: pH, °Brix, titratable acid, and citric acid by the spectrophotometric method described by Marier Boulet (1958) and protein by Bradford. The biomass analysis was by dry weight, at the end of the study.

The data obtained were analyzed with a univariate ANOVA, a Tukey test (p= 0.05). Also, a Pearson correlation test and according to the number of variables an AMOVA was determined and to know the influential variables a Principal Components Analysis was performed.

RESULTS AND DISCUSSION

The physicochemical analysis of the mango peel is presented in **Table 1**. The most acid peels were from the Piña and Manila varieties. The peels close to a pH value of 6.0 were from Coche, Tommy, and Criollo, with only the Ataúlfo having the highest value. All the varieties presented an ash percentage between 2.6 and 2.8. All varieties of mango fruit peel had an acidity value of less than 0.1 %, the lowest corresponding to pineapple mango fruit. Only the Manila and Ataúlfo varieties had higher brix values than the other varieties. In percentage of humidity, the Criollo and Tommy varieties had values higher than 30 %, the others had lower values. The Ataúlfo variety was the only one that presented lower humidity values.

The behavior dynamics of the pH values, brix degrees, titratable acidity, and protein during the 9 days of study are presented in **Figure 1**. The Tommy and Criollo varieties remained below the pH values of the control treatment **(Figure 1a)**. The other treatments were up to the 3rd day above the pH value of the control and from day 6, all the treatments presented a lower pH value than the control.

The brix degree values in all treatments gradually decreased **(Figure 1b)**, observing that after day 3 the reduction was slower and the values corresponding to the Ataúlfo variety did not reduce the reduction speed.

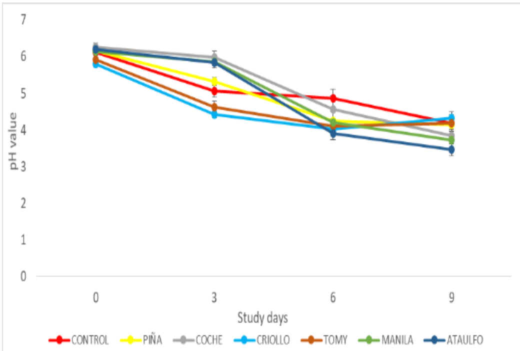
In the titratable acidity variable (calculated for lactic acid), **(Figure 1c)**, the treatments of the Criollo, Tommy, and Manila varieties, the highest concentration were observed on day 3 of the study. Where the tomy variety was the only one that presented the highest value of all the treatments. In the other treatments, the highest point of the acidity value occurred on the 6th day of the study.

The dynamics of soluble protein in the culture medium, allowed us to observe that the medium with flour of the Pineapple variety **(Figure 1d)**, presented the lowest concentration of soluble protein (Bazhenova, *et al*., 2021; Taufiqurrahman & Christyaningsih, 2021). On day 3 of the study, most of the treatments presented soluble protein values, higher than the control treatment. On day 6, the second treatment with the lowest concentration was that of the Criollo variety and the others were above that value. On the last day of the study, the medium with Tommy flour presented a lower protein value than the control, and those of the Manila and Ataúlfo varieties had a similar concentration to the control. Only in the medium with flour of the car variety, was it the only one that exceeded the protein value of the control.

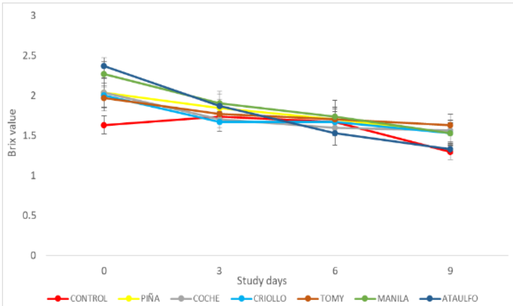
The statistical analysis allowed us to find that there were significant differences between the treatments for the variable pH (p=0.0006), brix (p=0.022), and highly significant for the variables titratable acidity (p<0.0001), and soluble protein (p<0.0001).

Table 1. Physicochemical analysis of the mango peel.

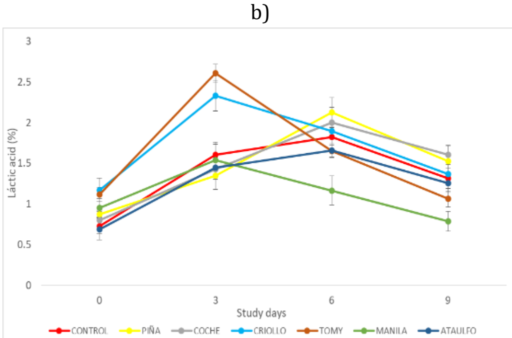
Variety	pH	Ash (%)	Titratable acidity (%)	Brix	Humidity (%)
Coche	5.7	2.6	0.07	0.84	29.8
Piña	5.4	2.7	0.05	0.78	28.3
Criollo	5.8	2.8	0.07	0.82	32.4
Tommy	5.8	2.6	0.09	0.80	32.4
Manila	5.5	2.6	0.07	0.90	27.2
Ataúlfo	6.0	2.8	0.09	0.93	22.6



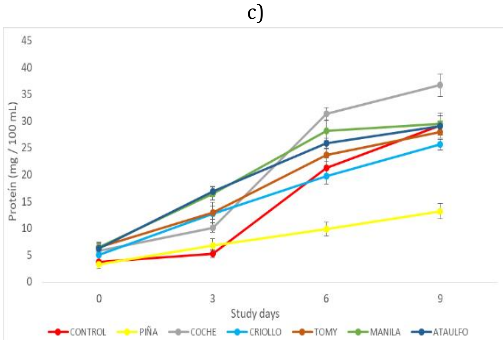
a)



b)



c)



d)

Figure 1. Dynamics of pH (a), Brix (b), acidity (c), and protein (d) during the growth of Penicillium sp, in the production of citric acid.

In what corresponds to the production of citric acid (AC), **(Figure 2)**, the culture media where flour of the pineapple and car varieties was added, the concentration of AC was lower than that obtained with the control treatment. The other treatments presented higher values in CA concentration than the control treatment. On day 3, the rest of the treatments presented similar values of CA and on day 6 there is a similarity in the dynamics of the Criollo and Ataulfo varieties and between the dynamics of the Manila and Tommy varieties. At the end of the study, the one that presented the highest value of AC was the one that received coffin flour.

The difference between treatments, on citric acid production, was highly significant (p<0.0001).

The *Penicillium* biomass production is presented in **Table 2**. The highest amount of biomass was obtained in the control treatment and the lowest biomass values occurred in the coffin treatment. The biomass values of the other treatments were between these two, mentioned above.

The correlation values between the studied variables, which were positive, are between brix degrees and pH values (r=0.81) and between citric acid concentration and soluble protein (r=0.80). The other correlations were negative, such as brix degrees and citric acid concentration (r=-0.71), between brix degrees and soluble protein (r=-0.69), between pH values and citric acid concentration (r=-0.84), between the pH values and the soluble protein (r=-0.78), all these values presented p<0.0001.

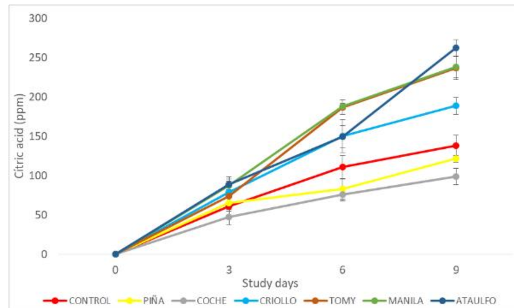


Figure 2. Dynamics of citric acid production with Penicillium sp.

Table 2. Biomass obtained from the growth of Penicillium sp, at the end of 9 days, for all varieties of mango fruit peel.

Variable	Fruit varieties						
	Piña	Coche	Criollo	Tommy	Manila	Ataúlfo	Control
Biomass (g)	0.096 ±0.001	0.082 ±0.001	0.103 ±0.002	0.076 ±0.001	0.077 ±0.001	0.064 ±0.001	0.196 ±0.002

A correlation was also made in the final concentration of citric acid and the analysis of the mango fruit peel, obtaining the following: between citric acid and pH it was r=0.50, between citric acid and brix degrees it was r=0.59 and between citric acid and the concentration of organic acids was r= 0.76.

The multivariate analysis called the Wilks value indicated that there were highly significant differences in all the variables, between treatments and between study days (p<0.0001). The comparison of the means between treatments **(Table 3)** indicated that the fermentation process of manila and ataúlfo were similar. The other treatments were significantly different. The principal component analysis distributed the samples of the treatments between two components, where component 1 covered 67.20% and component 2, was 21.20%. In component 1, the variables that governed the fermentation process were brix degrees, pH, concentration of citric acid, and soluble protein. Considering the second component of acidity. The cophenetic correlation value was 0.980.

Table 3. Hotelling test of the variables of the citric acid production process.

Treatment	Study variables					
	Brix	Ph	Acidity	Citric acid	Proteín	Hotelling
Control	1.58	5.08	1.37	77.28	0.15	B
Piña	1.78	4.96	1.43	67.30	0.08	A
Coche	1.73	5.21	1.43	55.44	0.21	D

Criollo	1.72	4.60	1.94	104.31	0.16	C
Tommy	1.77	4.63	1.81	124.26	0.18	E
Manila	1.86	5.03	1.06	128.21	0.20	F
Ataúlfo	1.78	4.93	1.24	124.86	0.20	F

The raw materials used as energy sources must be suitable to allow the growth of specific strains for the generation of products of industrial importance. According to Pérez et a., (2018) and Pérez-Navarro *et al*., (2016), the suitable substrates to be used in the production of citric acid, must have an adequate balance of mass and energy, considering that the common ones for the production of this molecule have been beets, orange peel, coffee peel, pumpkin residues, among others. According to Liu *et al*., (2023), Sorrenti *et al*., (2023), Linares-Luna *et al*., (2022), Magalhaes *et al*., (2023), and Corredor and Pérez, (2018), viable agricultural residues are candidates as raw material sources, must contain essential components such as fiber, carbohydrates, ashes, carotenes, proteins, organic acids, among others. In the analysis of the mango peel samples **(Table 1)**, of the different varieties used, they showed viable physicochemical characteristics to be used as raw material in the production of citric acid, observing that the pH, Brix, and acidity values coincide with the indispensable variables indicated. According to our results, these variables were important for obtaining citric acid at the end of the process **(Figure 2)**, indicating that the peel of the Criollo, Tommy, Manila, and Ataúlfo varieties can be considered as an alternative potential substrate to produce citric acid.

The pH values and brix degrees were reduced as the components of the culture medium with and without added flour were transformed, due to the growth of the *Penicillium* mycelium, reaching values less than 5 in pH and less than 2 in brix degrees, in each treatment studied. Similar behavior was observed in cocoa fermentation, as reported by Cubillos *et al*., (2019). Adeoye and Lateef *et al*., (2021) and Win *et al*., (2021), indicate that during the fermentation involved in the generation of citric acid, acidification occurs during the process, reaching values less than 5.0 and a consumption of soluble sugar. , close to 75 %, behavior similar to the present study with *Penicillium* using mango peel as an additional substrate **(Figures 1a and 1b)**.

The presence of maximum points of soluble organic acids (titratable acidity) present in the different culture media where mango flour was used, suggests two scenarios: a) that the soluble organic acids come from the mango peel flour, they were consumed as the fungus grew and citric acid (Najim *et al*., 2022) was synthesized and b) that during the growth of *Penicillium*, organic acids were generated as a product of the metabolism of fungal growth, being excreted into the medium and subsequently assimilated during 9 days of growth, being consumed in the three subsequent days to the maximum concentration reached, as the fungus grew and citric acid was produced. According to Xue *et al*., (2021), Odu *et al*., (2020), and Tong *et al*., (2019), during the metabolism of citric acid, there may be a reduction in pH together with a reduction in acidity titratable, being more accentuated when there is a high concentration of sugar. When the fermentation process is greater, there is more enzymatic activity and the consumption of sugars and organic acids is very accentuated in the biosynthesis of citric acid (Tong *et al*., 2019; Odu *et al*., 2020), what was previously exposed was similar. to what was observed in the production of citric acid when mango peel flour of the Tommy, Manila, and Ataúlfo varieties was included **(Figures 1c, 1d, and 2)**. According to Win *et al*., (2021), all parts of the fruits have essential components for microbial fermentation in the production of citric acid, the pulp and the process for obtaining these components being the most viable, according to Al-Shoaily *et al*., (2014), Adeoye and Lateef, (2021) and Win *et al*., (2021), together with the processing of the substrates used as nutrient sources, influences the yield of citric acid obtained. Given the above, it is possible that the drying of the shell to produce the flour and the grinding conditions, together with the casting of 1300 meshes to obtain a small particle size, favored the transfer of these components to the culture medium and allowed the good synthesis of citric acid, being higher in the Tommy, Manila, and Ataúlfo varieties.

CONCLUSION

The mango fruit peel of all the varieties presented pH values between 5.4 and 6.0, with brix degree values between 0.78 and 0.90, a humidity that oscillated between 22.6 and 32.4%, an amount of ash between 2.6 and 2.8%, and a titratable acidity that was presented between 0.05 to 0.09 %.

All the treatments showed lower values of pH 5.0 at the end of the study and in brix degree values, only the Ataúlfo treatment showed a linear reduction, presenting a value of 1.3 at the end, similar to the control.

The treatments of the varieties Tommy, Ataúlfo, and Manila were the only ones that had lower titratable acidity values than the control, being the only ones with the highest values of citric acid in the culture medium.

The treatment with the car variety was the only one that presented the highest values of soluble protein and where the lowest citric acid production was observed.

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