Research Article

Fermentation of Musa paradisiaca Peels to Produce Citric Acid

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Among organic acids, citric acid (CA) features the highest production volume and the greatest economic potential. The steadily increasing demand for CA necessitates the improvement and diversification of the corresponding production techniques via the incorporation of more environmentally friendly and less costly processes such as the bioconversion of agroindustrial by-products. Musa paradisiaca, known as plantain, is a food product of global importance; however, the related by-products are scarcely utilized. Herein, we investigate CA production from M. paradisiaca peels via fermentation with Aspergillus niger. Compositional analysis shows that the above peels contain 623 g·kg⁻¹ total carbohydrates, 374 g·kg⁻¹ starch, and 91 g·kg⁻¹ protein and therefore are rather rich in carbon, with other elements contained in substantial amounts corresponding to K (28 g·kg⁻¹), N (10 g·kg⁻¹), Fe (39 mg·kg⁻¹), Na (71 mg·kg⁻¹), Zn (16 mg·kg⁻¹), and Cu (18 mg·kg⁻¹). Evaluation of solid-substrate fermentation conditions (pH and inoculum loading) reveals that CA production is maximized (29 g·kg⁻¹) at 10% consistency, 30°C, pH 1.4, and inoculum loading = 20 mg, demonstrating that pH is the most important parameter determining fermentation efficiency. As a result, M. paradisiaca peels are concluded to be a suitable substrate for CA biosynthesis via fermentation with A. niger under optimal nutritional conditions.

1. Introduction

Citric acid (CA; 2-hydroxypropane-1,2,3-tricarboxylic acid), one of the most important and versatile among the currently produced organic acids, is widely used in food, cosmetics, agricultural, pharmaceutical, chemical, and metallurgical industries [1–3] because of its antioxidant, pH-regulating, acidulant, and metal ion-sequestering properties. The worldwide commercial demand for CA in 2017 equaled 1,977 kilotons [4] and is expected to increase by 3.5–4% every year [5].

CA is largely obtained through fermentation by fungi, bacteria, and yeasts [6], e.g., species of the genera Aspergillus, Citromyces, Penicillium, Monilia, Candida, Pichia, Arthrobacter, Trichoderma, Bacillus, and Corynebacterium spp. and yeasts such as Candida tropicalis, C. oleophila, C. guilliermondii, C. citroformans, Hansenula anamola, and Yarrowia lipolytica [7–10]. Aspergillus species, especially A. niger, are the ones most widely used for this type of production, with the most effective ones characterized by low activities of isocitrate dehydrogenase and aconitase hydratase and a high activity of citrate synthetase [11, 12].

Industrially, CA is produced by fermentation of sucrose-containing media such as cane and beet molasses [6]. However, some studies have shown that CA can also be produced by solid-state fermentation (SSF) of low-cost agroindustrial by-products such as apple pulp, banana peel, cassava bagasse, coffee husk, corn cob, mango peel, orange peel, papaya peel, pineapple waste, sugar cane bagasse, and others [12–26] (Table 1).

Agroindustrial by-products represent one of the most abundant and potentially more valuable materials on the planet. They constitute a renewable resource from which many useful biological and chemical products can be derived [27–31]. The agroindustrial waste produced globally is scarcely used, and much of it remains as waste in the environment. For example, thousands of tons of plantain (Musa paradisiaca) and banana (Musa sapientum) peel are...
produced annually in the tropics, which is a nuisance to the environment [32, 33]. These wastes can be microbially degraded and simultaneously converted into novel materials or new products for industrial applications. Recently, banana peel has been utilized for various applications [34–37], including CA production. Kareem and Rahman [14] reported a high production of CA (82 g·kg⁻¹ dry weight) after 96 h of growth at 30°C in the presence of methanol and copper ions. On the other hand, plantain peels have not been evaluated much for industrial applications, and one of the few studies investigated it as a source of dietary fiber and antioxidant compounds [38]. Nevertheless, plantains are one of the important food crops in many developing nations and are grown in more than 100 countries in the tropics and subtropics [39], including Panama, where it is of great gastronomic importance because of its high starch and potassium contents. The Panamanian industry produces and commercializes packaged fried plantains, leaving a large amount of peels with potential industrial application as wastes. Thus, this agroindustrial by-product can be converted into a variety of chemicals and value-added products such as CA through the fermentation process.

Notably, the production of CA by fermentation is strongly influenced by carbon-source type and concentration, pH, nitrogen and phosphorus limitations and trace element-controlled concentrations [6, 10]. One of the important advantages of SSF is that it obviates the large requirements of nitrogen and phosphorus due to the lower diffusion rates of nutrients from the substrate. In addition, unlike in submerged fermentation, the presence of trace elements in the material does not affect CA production in the SSF process [6, 12, 40]. Studies on CA production by a SSF system showed no such influence despite the high concentrations of metal ions in the substrate. In contrast, supplementation with additional amounts of mineral ions increased the production of CA by 1.4–1.9 times [41].

The search for low-cost and easily accessible raw materials that can be used as fermentable substrates (C sources) is currently one of the most interesting challenges of biotechnology. There are several agroindustrial substrates of great potential that can be used for the production of CA, such as *Musa paradisiaca* (plantain) peel, a substrate with a high potential for obtaining various substances.

In Panama, plantain peels are not utilized for any particular purpose and are therefore promising raw materials for the production of industrially valuable chemicals. Therefore, we herein investigate CA production by fermentation of *M. paradisiaca* peel with *A. niger* and establish optimal operation conditions. This is one of the first works on the use of this substrate for obtaining CA by SSF.

### Table 1: Citric acid production by solid-state fermentation of low-cost agroindustrial by-products.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Organism</th>
<th>Fermentation conditions</th>
<th>Citric acid content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple pomace</td>
<td><em>A. niger</em> van Tieghem MTCC 281</td>
<td>30°C and 120 h</td>
<td>46 g·kg⁻¹</td>
<td>Kumar et al. [13]</td>
</tr>
<tr>
<td>Banana peel</td>
<td><em>A. niger</em> UABN 210</td>
<td>30°C, 96 h, 10 g·L⁻¹ methanol, and 10 ppm copper ions</td>
<td>82 g·kg⁻¹</td>
<td>Kareem and Rahman [14]</td>
</tr>
<tr>
<td>Cassava peel</td>
<td><em>A. niger</em> mutant strain</td>
<td>30°C, 0.5% consistency, pH 4.5, 6% inoculum, and 84 h</td>
<td>89 g·L⁻¹</td>
<td>Adeoye et al. [15]</td>
</tr>
<tr>
<td>Coffee husk</td>
<td><em>A. niger</em></td>
<td>30°C, pH 4.5, 72 h, and 50% moisture content</td>
<td>150 g·kg⁻¹</td>
<td>Shankaranand and Lonsane [16]</td>
</tr>
<tr>
<td>Corn cob</td>
<td><em>A. niger</em></td>
<td>28°C, 144 h, 150 g·kg⁻¹ sucrose, and 50% consistency</td>
<td>138 g·kg⁻¹</td>
<td>Addo et al. [17]</td>
</tr>
<tr>
<td>Corn grains</td>
<td><em>A. niger</em> ATCC 9142</td>
<td>25°C, 240 h, and 82% moisture content</td>
<td>6 g·kg⁻¹</td>
<td>Xie and West [18]</td>
</tr>
<tr>
<td>Coconut husk</td>
<td><em>A. niger</em> KLCN2</td>
<td>30°C, 168 h, and 55% moisture content</td>
<td>128.6 g·kg⁻¹</td>
<td>Lingappa et al. [19]</td>
</tr>
<tr>
<td>Mango peels</td>
<td><em>A. niger</em></td>
<td>32°C, pH 5, 192 h, and 11% consistency</td>
<td>30 g·L⁻¹</td>
<td>Abbas et al. [20]</td>
</tr>
<tr>
<td>Oat bran</td>
<td><em>A. niger</em></td>
<td>28°C, pH 4.5, 72 h, 20% fructose, and 2.5 g·L⁻¹ NH₄NO₃</td>
<td>62 g·kg⁻¹</td>
<td>Rao and Reddy [21]</td>
</tr>
<tr>
<td>Orange peel</td>
<td><em>A. niger</em> CECT-2090</td>
<td>30°C, 84 h, and 70% moisture content</td>
<td>193 g·kg⁻¹</td>
<td>Torrado et al. [22]</td>
</tr>
<tr>
<td>Sweet orange peels</td>
<td><em>A. niger</em></td>
<td>32°C, pH 4, 144 h, and 11% consistency</td>
<td>11 g·L⁻¹</td>
<td>Abbas et al. [20]</td>
</tr>
<tr>
<td>Pineapple waste</td>
<td><em>A. niger</em> KS-7</td>
<td>30°C, 120 h, and 65% moisture content</td>
<td>6 g·kg⁻¹</td>
<td>Kareem et al. [23]</td>
</tr>
<tr>
<td>Sugar cane bagasse</td>
<td><em>A. niger</em></td>
<td>30°C, pH 5, and 65% moisture content</td>
<td>3.5 g·kg⁻¹</td>
<td>Sharan et al. [24]</td>
</tr>
<tr>
<td>Beet root pulp waste</td>
<td><em>A. niger</em></td>
<td>30°C, pH 5, 65% moisture content</td>
<td>3.4 g·kg⁻¹</td>
<td></td>
</tr>
<tr>
<td>Guava pulp waste</td>
<td><em>A. niger</em></td>
<td>30°C, pH 5, 65% moisture content</td>
<td>4.8 g·kg⁻¹</td>
<td></td>
</tr>
<tr>
<td>Indian jujube pulp waste</td>
<td><em>A. niger</em></td>
<td>30°C, pH 5, 65% moisture content</td>
<td>4 g·kg⁻¹</td>
<td></td>
</tr>
<tr>
<td>Papaya pulp waste</td>
<td><em>A. niger</em> ATCC 16404</td>
<td>Room temperature (min 22°C–max 32°C), 192 h, and 6% moisture content</td>
<td>3.8 g·kg⁻¹</td>
<td>Bezalwar et al. [25]</td>
</tr>
<tr>
<td>Pineapple pulp waste</td>
<td><em>A. niger</em></td>
<td>30°C, pH 5, 65% moisture content</td>
<td>5 g·kg⁻¹</td>
<td></td>
</tr>
<tr>
<td>Wood apple pulp waste</td>
<td><em>A. niger</em></td>
<td>30°C, pH 5, 65% moisture content</td>
<td>5.5 g·kg⁻¹</td>
<td></td>
</tr>
<tr>
<td>Grape residues</td>
<td><em>A. niger</em></td>
<td>30°C, pH 5, 65% moisture content</td>
<td>5.5 g·kg⁻¹</td>
<td></td>
</tr>
<tr>
<td>Mosambi peel</td>
<td><em>A. niger</em></td>
<td>30°C, pH 2.1–3.1, after 24 h, 40 g·L⁻¹ methanol, and 70% moisture content</td>
<td>34.4 g·kg⁻¹</td>
<td>Goud et al. [26]</td>
</tr>
<tr>
<td>Sugar cane bagasse</td>
<td><em>A. niger</em></td>
<td>30°C, pH 2.1–3.1, after 24 h, 40 g·L⁻¹ methanol, and 70% moisture content</td>
<td>28.6 g·kg⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24.9 g·kg⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

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*These substrates beet root pulp waste, guava pulp waste, Indian jujube pulp waste, papaya pulp waste, pineapple pulp waste, wood apple pulp waste, belong to the reference Bezalwar et al. [25]. The substrates grape residues, mosambi peel, sugar cane bagasse belong to the reference Goud et al. [26]*
2. Materials and Methods

2.1. Raw Materials and Chemicals. Samples of *M. paradisiaca* peels provided by a local supplier were dried for 48 h at 50°C, milled, and stored in plastic bags under dry conditions until use. All chemicals were of analytical grade.

2.2. Compositional Analysis. Samples were analyzed for starch, total carbohydrate, reducing sugar, protein, and mineral contents. All analyses were performed in triplicate. Total carbohydrate and starch contents were determined by the phenol-sulfuric acid method. For total carbohydrate quantitation, a test tube containing the sample (0.1 g) and HCl (5 mL, 2.5 N) was heated in a water bath at 95°C for 3 h. The resulting suspension was neutralized with Na₂CO₃, diluted with water (5 mL), and centrifuged. The dilution/centrifugation process was repeated four more times. The supernatant volume was adjusted to 100 mL with water, and a 0.1 mL aliquot of this solution was treated with 50 g·L⁻¹ aqueous phenol (1 mL) and concentrated 96% H₂SO₄ (5 mL), vortexed for 1 min, and heated at 30°C for 20 min in a water bath. For starch content determination, a dispersion of the sample (0.1 g) in hot 800 mL⁻¹ ethanol (8 mL) in a test tube was stirred for 1 min and then centrifuged at 3500 rpm for 10 min. The supernatant was removed, and the dispersion/centrifugation/supernatant removal process was repeated three more times. The residue was extracted with a mixture of water (5 mL) and HClO₄ (6.5 mL) at 4°C for 20 min, treated with water (20 mL), and centrifuged. The extraction-dilution-centrifugation process was repeated two more times. The combined extracts were diluted to 100 mL with water, and a 0.1 mL aliquot of the obtained solution was treated with 50 g·L⁻¹ aqueous phenol (1 mL) and concentrated 96% H₂SO₄ (5 mL), vortexed for 1 min, and then centrifuged at 30°C for 20 min in a water bath. Finally, total carbohydrate and starch contents were determined by visible-light spectrophotometry at a wavelength of 490 nm using a calibration curve prepared with glucose as a standard.

Reducing sugars were quantified by visible-light spectrophotometry at a wavelength of 540 nm using the dinitrosalicylic acid (DNS) method [42]. Typically, a suspension of the sample (0.1 g) in hot 800 mL⁻¹ ethanol (8 mL) contained in a test tube was heated in a water bath at 95°C for 10 min and then centrifuged at 2500 rpm for 5 min. The supernatant was collected, ethanol was evaporated in a water bath at 80°C, and the obtained residue was treated with water (10 mL). A 0.7 mL aliquot of this solution was transferred into a test tube and treated with the DNS reagent (1.5 mL). The resulting mixture was heated in a boiling water bath for 5 min and then brought to room temperature in a water bath held at 25°C. Finally, the content of reducing sugars was determined from the absorbance of the cooled mixture at 540 nm using a calibration curve prepared with glucose as a standard. DNS reagent was prepared by dissolving 0.748 g dinitrosalicylic acid, 0.564 g crystalline phenol, 21.612 g Rochelle salt, 1.412 g NaOH, and 0.564 g sodium sulphite in 100 mL.

The total nitrogen concentration was determined by the Kjeldahl method (AOAC 976.05) [43]. For mineral content determination, the ashed sample was dissolved in hydrochloric acid, and the resulting solution was analyzed by atomic absorption spectroscopy according to AOAC Method 984.27.

2.3. Growth of the A. niger Strain and Preparation of the Corresponding Inoculum. The employed *A. niger* ATCC 6275 strain was conserved in Petri dishes with 40 g·L⁻¹ potato dextrose agar incubated at 30°C. To obtain the inoculum, spores were harvested from PDA cultures after seven days of growth using a 1 g·L⁻¹ Tween 80 solution. Spore counting was carried out in a Neubauer chamber until the required final concentration of 10⁶ to 10⁸ mL⁻¹ was obtained. The resulting suspensions were used as inoculum. The inoculum mass was determined by gravimetry, in which a determined volume of the spore suspension was placed on a filter paper, the remaining residue was dried at 105°C for 1 h, and the dry weight was calculated.

2.4. Solid-State Fermentation. Fermentation was carried out at 10% consistency in an Erlenmeyer flask (125 mL) as a fermenter. The fermenter was charged with the sample (5 g, dry base), a nutrient solution previously autoclaved at 121°C for 15 min (50 mL; NH₄Cl (1 g·L⁻¹), KH₂PO₄ (3 g·L⁻¹), and MgSO₄·7H₂O (1.5 g·L⁻¹)) [44], and the inoculum. The effects of pH (1.4–5.6) and inoculum loading (8–37 mg) were evaluated by a central composite design to optimize CA yield (Tables 2 and 3). The reaction mixture was incubated at 30°C and 150 rpm for eight days, and CA content was quantified every 24 h. Additionally, fermentation was evaluated for a wet substrate (70% humidity) previously supplemented with the abovementioned nutrient solution. The flask containing the wet substrates was supplemented with the inoculum (15 mg) and incubated at 30°C and 150 rpm for 10 days, with CA quantitation performed every 48 h. After fermentation, 25 mL water was added to the flask and it was agitated for 10 min on a rotary shaker to extract CA. The mixture was filtered through a Whatman filter paper no. 41, and the supernatant was used for the estimation of CA.

2.5. CA Determination. The produced CA was quantified using the pyridine-acetic acid spectrophotometric method. Typically, an aliquot of filtered fermentation broth (500 μL) was placed in a test tube, treated with glacial acetic acid (4 mL), and heated at 60°C for 10 min in a water bath. Subsequently, pyridine (500 μL) was added, and heating was continued for another 40 min. The reaction mixture was allowed to cool for 10 min, and CA was quantified by visible-light spectrophotometry at a wavelength of 420 nm using a calibration curve constructed with CA as a standard. All determinations were carried out in duplicate.

2.6. Statistical Analysis. To determine the conditions required for maximum CA production by the fermentation process, the variables, pH and inoculum loading, were studied. The influence of each variable was determined by response surface methodology (RSM) [45]. The model was
based on a central composite circumscribed design consisting of a factorial design 2^3 and star points. The variable values were coded and normalized in unitary values: −1 was defined as the lowest value of a variable and +1 was defined as the highest value. From the extreme variable values, the central point (coded 0) was set and assayed in triplicate for calculation of errors in the experiments. Four star points distributed at a distance of 1.41 from the central point were included. Hence, 11 design points were used in total. The complete experimental design is presented in Table 2.

A second-order function that best describes the system’s behavior was determined by a multiple linear regression method (MLR). The statistical validation was performed by a one-way ANOVA test with 95% confidence level. The optimal condition values were determined based on the response surface calculated using the SIMPLEX method [45]. All the calculations were performed with the software Modde 12.0 (Umetrics, USA).

### 3. Results and Discussion

#### 3.1. Chemical Composition of M. paradisiaca Peel

The total carbohydrate, starch, reducing sugar, and protein contents of plantain peel were determined as 623 ± 20, 374 ± 2, 33 ± 3, and 91 ± 1 g·kg⁻¹, respectively, which showed that this material was rather rich in carbon and protein. Similar results have been reported by other authors. For example, Anchundia et al. [46] determined the content of starch in edible films based on M. paradisiaca peel as 380 g·kg⁻¹, while Monsalve et al. [47] reported that this peel contained 400 g·kg⁻¹ starch. Agama-Acevedo et al. [38] and Emaga et al. [48] determined the protein contents of Musa paradisiaca and Musa spp. peels as 10 and 80–100 g·kg⁻¹, respectively.

The contents of N, P, K, Ca, and Mg in M. paradisiaca peel were determined as 10, 0.7, 28, 0.4, and 0.6 g·kg⁻¹, respectively, while those of Fe, Cu, Mn, Zn, and Na were determined as 38.6, 17.6, 0.15, 15.6, and 71.2 mg·kg⁻¹, respectively. The content of P (required for ATP/ADP formation [49]) that is needed for CA production has been estimated as 0.5–5 g·L⁻¹ [6], and a K content of 0.28 g·L⁻¹ has been shown to result in enhanced CA production [49]. The level of N required for CA production ranges between 0.1 and 0.4 g·L⁻¹ [12] and has a great effect on the production of CA because this element is found in proteins and contributes to the development of cellular metabolism [23]. However, high nitrogen levels increase the consumption of sugars and the growth of fungi, decreasing CA production [50]. Finally, optimal Mg levels have been determined as 0.19 g·L⁻¹-Mg²⁺ [51]. Although the levels of the above macronutrients in the raw material utilized herein were high, the corresponding levels in fermentation mixtures were low and substrate supplementation was therefore required.

On the contrary, certain trace elements such as Zn, Mn, Fe, and Cu are also important for CA production [6, 52]. For example, the optimal levels of Fe and Zn for CA production have been determined as 1.3 and 0.3 mg·L⁻¹, respectively [7]. Kareem and Rahman [14] showed that the presence of Fe and Mn has a detrimental effect on the accumulation of CA, whereas the opposite was true for Zn and Cu. Since the contents of these trace elements in M. paradisiaca peels were higher than optimal, fermentation experiments were performed under two conditions, namely, with the solid substrate at 10% consistency and with a wet solid substrate (70% moisture, 30% consistency), both of which were previously supplemented with a nutrient solution. Nevertheless, one of the great advantages of solid-substrate fermentation is that trace elements may not negatively affect CA production, contrary to the case of submerged fermentation, when microelements can dissolve in the medium [6, 12, 40].

#### 3.2. CA Production

The maximal levels of CA in mixtures fermented at 10% consistency were observed on the fourth day (96 h) (Figure 1) and ranged from 7.9 to 29 g·kg⁻¹ dry

### Table 2: Experimental design for fermentation of M. paradisiaca peels.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coded levels</th>
<th>pH</th>
<th>Inoculum loading (mg)</th>
<th>CA (g·kg⁻¹) experimental</th>
<th>CA (g·kg⁻¹) calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−1.41 -1 0 1 1.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>1.4 2 3.5 5 5.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculum loading (mg)</td>
<td>3 8 20 32 37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Effects of pH and inoculum loading on CA production.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>pH (code level)</th>
<th>Inoculum loading (mg) (code level)</th>
<th>CA (g·kg⁻¹) experimental</th>
<th>CA (g·kg⁻¹) calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 (−1)</td>
<td>8 (−1)</td>
<td>17.8 ± 0.5</td>
<td>19.3</td>
</tr>
<tr>
<td>2</td>
<td>5 (1)</td>
<td>8 (−1)</td>
<td>11.5 ± 0.4</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>2 (−1)</td>
<td>32 (1)</td>
<td>19.0 ± 0.8</td>
<td>21.5</td>
</tr>
<tr>
<td>4</td>
<td>5 (1)</td>
<td>32 (1)</td>
<td>7.9 ± 0.4</td>
<td>7.7</td>
</tr>
<tr>
<td>5</td>
<td>1.4 (−1.41)</td>
<td>20 (0)</td>
<td>29 ± 1</td>
<td>26.4</td>
</tr>
<tr>
<td>6</td>
<td>5.6 (1.41)</td>
<td>20 (0)</td>
<td>8.9 ± 0.4</td>
<td>10.2</td>
</tr>
<tr>
<td>7</td>
<td>3.5 (0)</td>
<td>3 (−1.41)</td>
<td>11 ± 1</td>
<td>11.1</td>
</tr>
<tr>
<td>8</td>
<td>3.5 (0)</td>
<td>37 (1.41)</td>
<td>12.3 ± 0.7</td>
<td>10.9</td>
</tr>
<tr>
<td>9</td>
<td>3.5 (0)</td>
<td>20 (0)</td>
<td>11 ± 2</td>
<td>12.3</td>
</tr>
<tr>
<td>10</td>
<td>3.5 (0)</td>
<td>20 (0)</td>
<td>11 ± 2</td>
<td>12.3</td>
</tr>
<tr>
<td>11</td>
<td>3.5 (0)</td>
<td>20 (0)</td>
<td>14 ± 2</td>
<td>12.3</td>
</tr>
</tbody>
</table>
material base (Table 3). Analysis of experimental data allowed CA concentration to be expressed in terms of pH as

$$CA_{\text{concentration}} \left( \text{g} \cdot \text{kg}^{-1} \right) = 12.3 \pm 3.3 - 5.7$$

$$\pm 2.0 \text{pH} + 3.0 \pm 2.4 \text{pH}^2.$$  \hspace{1cm} (1)

This second-degree polynomial, which was validated by an ANOVA test to 95% confidence ($p < 0.05$; the model was found to be statistically significant) (Table 4), demonstrates that CA concentration was only affected by pH ($p = 0.0008$) while inoculum loading did not have a marked effect ($p = 0.93$) (Table 5). According to this equation, CA concentration increased with decreasing pH. Additionally, no interaction was detected between pH and inoculum concentration ($p = 0.33$). The error values corresponded to a confidence level of 95%. The variables in the quadratic polynomial were scaled and centered. Based on the experimental conditions used in the experimental design, the polynomial was used to predict CA production (Table 1), and values predicted by the model were close to the experimental values ($R^2 = 0.92$).

The effects of the fermentation conditions at 10% consistency are shown in the surface contour plot of Figure 2 while the influence of pH on CA concentration is shown in Figure 3. Since pH strongly affected the fermentation process, CA production was evaluated at different pH values. As a result, optimal pH was determined as 1.4, in agreement with the findings of Papagianni [7] and Max et al. [52], who revealed that CA production should be performed at pH ≤ 2. Under these conditions, the production of other organic acids such as gluconic and oxalic acids is inhibited, which increases the yield of CA and reduces the risk of contamination by other microorganisms.

For wet-substrate fermentation, the maximal CA level (20 g·kg⁻¹ dry material base) was observed on the sixth day (144 h) (Figure 4). Although the humidity level used can help to promote exchange and diffusion processes, the obtained value was lower than those for fermentation at
10% consistency. This cannot be attributed to the presence of minerals because of the absence of free liquid for dissolution.

As the incubation temperature plays an important role in the production of CA, in this work, a temperature of 30°C was employed since it is the optimal temperature reported in most of the works published on the production of CA by fermentation with *A. niger* from different substrates (see Table 1). Above 35°C, the formation of CA is inhibited due to the higher production of derived acids and the inhibition of microbial growth [53].

Similarly, remarkably higher and even lower CA levels have been reported for other substrates (Table 1), e.g., Bezalwar et al. [25] investigated the production of CA from pineapple pulp, beet root waste, guava pulp, and papaya, achieving maximal CA concentrations of 5, 3.4, 4.8, and 3.8 g·kg⁻¹, respectively, after 192 h. Goud et al. [26] investigated CA production from sugar cane bagasse, grape residues, and mosambi peels, achieving maximal levels of 29, 34, and 25 g·kg⁻¹, respectively, while Kareem et al. [23] realized a CA level of 60 g·kg⁻¹ (after 120 h) by fermentation of pineapple peels in the presence of methanol.

**4. Conclusions**

CA was successfully produced from *M. paradisiaca* peels, with higher CA levels reached for a solid substrate immersed into a solution than for a wet substrate, possibly because the operational parameters such as pH and temperature could be better controlled in the former case. Thus, *M. paradisiaca* peels were concluded to be a promising low-cost fermentable substrate for obtaining high added-value products such as CA.

**Data Availability**

The data used to support the findings of this study are included within the article.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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**References**


