Multivariate Study and Analysis of the Production of Citric Acid from Dates by Surface Method

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Abstract

The production of citric acid from dates of the date palm Phoenix dactylifera was studied using a full factorial design with two design variables; Aspergillus niger strain, and starter juice treatment. Analyses of data have shown that mould type and the interaction between Aspergillus niger strain + juice treatment had significant effect ($P \geq 0.05$) on the final total acidity. It was also found that all design variables and the interaction between them had significant effect ($P \geq 0.05$) on the final pH and Total Soluble Solids. The highest total acidity expressed as citric acid was achieved with a commercial strain of Aspergillus niger, and filtered and centrifuged date juice.

Keywords

Dates; Citric; Aspergillus niger

1. Introduction

Dates continue to be the most abundantly produced fruit in Oman; the annual total yield according to ministry of agriculture and fisheries (MAF) Oman 2010 estimation was 276,405 tons. Although potentially there is very valuable raw material for many industries, the almost exclusive use of dates is still direct consumption. The high content of monosaccharides (glucose, and fructose) (Al-Farsi, Alasalvar et al. 2007) [1] makes the dates suitable for fermentation to produce various organic acids such as acetic acid and citric acid.

Citric acid is an organic acid that naturally occurs in fruits such as lemons, oranges, and may be synthesised from glycerol (Kristiansen Bjorn, Mattey et al. 1999) [2]. It is also produced via fermentation by microorganisms action, many microorganisms accumulate citric acid, among them is Aspergillus niger, A. awamori, Absidia sp, Acremonium sp. ...etc. Nowadays Aspergillus niger is almost exclusively used to produce citric acid at industrial scale (Kristiansen Bjorn, Mattey et al. 1999) [2]. Citric acid has a wide range of use in food and beverage, in preparation of numerous industrial products, pharmaceuticals, and as a cleaning agent (Lotfy Walid A, Ghanem Khaled M. et al. 2007) [3]. Factorial experimental design and multivariate analysis is a useful tool used

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to estimate the impact a parameter (a design variable) has on a response (non design variable), this design is used for screening purpose to study main effects and interactions between combinations between design variables (Esbensen, Guyot et al. 2004) [4].

In the work reported here, we attempt to study the effects of two design variables (juice treatment, and mould type) on three non design variables (pH, total acidity, and total soluble solids TSS) in an experiment to produce citric acid by surface method from date juice.

2. Materials and Method

2.1. Microorganism

Two mould types were used in this study: a commercial strain of Aspergillus niger supplied by Culti-loops (the code is kept), and the other is a local strain of Aspergillus niger isolated from local dates surface.

2.2. Inoculum

Loops from pre prepared pure slants of Aspergillus niger (local or commercial) were streaked (Figure 1) on potato dextrose agar (Supplied by Himedia-India), and used to inoculate 18°brix date juice, then placed in a shaker incubator at 30˚C for 48 hours to produce pellets (Abid M. Al-, Al-Amri M. et al. 2010) [5].

2.3. Preparation of Date Juice

Dates sugars were extracted with water as explained in (Abid M. Al-, Al-Amri M. et al. 2010) [5], the final TSS was adjusted at 18°brix. The juice was subsequently treated in one of two filtration ways:

1) Filtration with whatman No.1 filter paper or
2) Filtration with whatman No.1 filter paper followed by centrifugation at 4000 RPM.

2.4. Inoculation with Aspergillus niger

Two types of Aspergillus niger, a commercial strain, and a local strain isolated from the surface of dates were used to inoculate the date juice.

2.5. Treatment with Tricalcium Phosphate (TP)

To eliminate inhibition caused by metals the date juice was treated with Tricalcium Phosphate Treatment to chelate minerals as explained in (Roukas T. and P.) [6] and (Abid M. Al-, Al-Amri M. et al. 2010) [5].

2.6. Analytical Methods

Total acidity was by titration with NaOH, pH was measured with pH meter (WTW, supplied by, Germany), TSS by bench top refractometer BS supplied by Stanley & Bellingham, UK.

2.7. Experimental Design

A full factorial design was implemented with 2 design variables and 3 non design variables as shown in Figure 2 & Table 1, the treatments were performed in triplicates:

2.8. Data analysis

All analysis of data were performed with MINITAB14.

3. Results and Discussions

Total acidity, pH, and total soluble solids were monitored daily in order to determine the pattern at which the changes occur. Acidity of fermenting medium was used as an indication of acid production. The total acidity increased slowly up to day eight then exponentially up to day 14 and leveled (Figure 3).
Figure 1. Aspergillus niger growth on PDA.

Table 1. Design variables (factors) and non design variables used.

<table>
<thead>
<tr>
<th>Design variables</th>
<th>Non design variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Filtration treatment:</td>
<td>1) pH</td>
</tr>
<tr>
<td>a) Filtration through whatman filter(T1).</td>
<td>2) Total soluble solids TSS</td>
</tr>
<tr>
<td>b) Centrifugation at 4000 RPM (T2).</td>
<td>3) Total acidity.</td>
</tr>
<tr>
<td>2) Inoculum type:</td>
<td></td>
</tr>
<tr>
<td>a) Local strain Aspergillus niger (S1).</td>
<td></td>
</tr>
<tr>
<td>b) Commercial strain Aspergillus niger (S2).</td>
<td></td>
</tr>
</tbody>
</table>

3.1. Optimization of Fermentation Conditions

A full factorial design was implemented as shown in Figure 2 & Table 1, this design is used for screening design variables (juice treatment and Aspergillus niger strain) in order to find out about main effects they have on non design variables or responses (pH, total acidity, and total soluble solids TSS) of the final product, in addition to interaction effects of the design variables (Esbensen, Guyot et al. 2004) [4].

3.2. Effect of Mould Type on Final Total Acidity

The final analysis results are shown in Table 2. The mould type significantly affected the total acidity ($P \geq 0.05$), total acidity increased from 0.37, to 3.75, and from 1.18 to 3.58% by changing the mould type from local (S1), to commercial (S2) strains respectively.

3.3. Effect of Mould Type on Final pH

The final pH was significantly affected by mould type ($P \geq 0.005$), the final pH (Figure 4) was reduced from 3.74, to 2.57, and from 2.9 to 2.58 by changing the mould type from local (S1), to commercial (S2) strains respectively.

3.4. Effect of Mould Type on Final TSS

The final total soluble solids TSS of the final product was used as an indication of sugar assimilation by Aspergillus niger (Figure 5). The mould type significantly ($P \geq 0.005$), affected the TSS. The readings of TSS of the final product Table 2 show decrease in TSS values from 14 to 13.8, and from 15.5 to 13.03 with change of mould type from local (S1), to commercial (S2) strains respectively.

3.5. Effect of Juice Treatment on Final Total Acidity

As can be seen in Table 3 the juice treatment had insignificant effect alone on total acidity ($P \geq 0.05$), but the interaction with the mould type was significant ($P \geq 0.05$) (see Table 3).
Figure 2. A process flow diagram showing design variables and non design variables.

Figure 3. Changes in acidity of fermented medium. T₁S₁ juice treatment 1 and mould type 1; T₁S₂ juice treatment 1 and mould type 2; T₂S₁ juice treatment 2 and mould type 1; T₂S₂ juice treatment 2 and mould type 2.

Table 2. Final results of acidity, pH, and TSS.

<table>
<thead>
<tr>
<th></th>
<th>% Acidity</th>
<th>pH</th>
<th>TSS °brix</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁S₁</td>
<td>0.37</td>
<td>3.74</td>
<td>14.00</td>
</tr>
<tr>
<td>T₁S₂</td>
<td>3.75</td>
<td>2.57</td>
<td>13.80</td>
</tr>
<tr>
<td>T₂S₁</td>
<td>1.18</td>
<td>2.90</td>
<td>15.50</td>
</tr>
<tr>
<td>T₂S₂</td>
<td>3.58</td>
<td>2.58</td>
<td>13.03</td>
</tr>
</tbody>
</table>
3.6. Effect of Juice Treatment on Final pH

The final pH was significantly affected by juice treatment ($P \geq 0.005$) (see Table 3), the final pH was reduced from 3.74, to 2.57, and from 2.9 to 2.58 Table 2 by changing the juice treatment from local (T1), to centrifugation (T2) respectively.

3.7. Effect of Juice Treatment on Final TSS

The readings of TSS of the final product (Table 2) show decrease in TSS values from 14 to 13.8, and from 15.5 to 13.03 with change of juice filtration treatment from (T1), to (T2) respectively. The decrease was significant ($P \geq 0.005$), which shows the need for thorough filtration technique.

3.8. Analysis of Variance (One Way ANOVA)

Analysis of variance (one way ANOVA) Table 4 shows that both parameters (main effects) used were significant, further more the interaction between mould type and juice treatment was significant as well.

4. Conclusion

The results revealed the significance of mould type and filtration technique in the production of citric acid from date juice. Despite the result the mould was more significant ($P \leq 0.005$) than juice treatment ($P \geq 0.05$); the combined effect of interaction between the two was significant ($P \leq 0.05$). The maximum total acidity achieved
Table 3. P values for non design variables versus design variables.

<table>
<thead>
<tr>
<th>Non design variables (responses)</th>
<th>Design variable (factors)</th>
<th>Total acidity</th>
<th>pH</th>
<th>TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger strain</td>
<td></td>
<td>0.000 (**)</td>
<td>0.000 (**)</td>
<td>0.020 (*)</td>
</tr>
<tr>
<td>Juice treatment</td>
<td></td>
<td>0.810 (NS)</td>
<td>0.000 (**)</td>
<td>0.005 (*)</td>
</tr>
<tr>
<td>2 way interaction: Aspergillus niger strain + Juice treatment</td>
<td></td>
<td>0.041 (*)</td>
<td>0.000 (**)</td>
<td>0.020 (*)</td>
</tr>
</tbody>
</table>

(**) $P \leq 0.001$, (*) $P \leq 0.05$, (NS) not significant.

Table 4. Analysis of variance (one way ANOVA).

<table>
<thead>
<tr>
<th>Non design variables (responses)</th>
<th>Design variable (factors)</th>
<th>Total acidity</th>
<th>pH</th>
<th>TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main effects</td>
<td></td>
<td>0.000 (**)</td>
<td>0.000 (**)</td>
<td>0.004 (*)</td>
</tr>
<tr>
<td>2 way interaction</td>
<td></td>
<td>0.041 (**)</td>
<td>0.000 (**)</td>
<td>0.020 (*)</td>
</tr>
</tbody>
</table>

(**) $P \leq 0.001$, (*) $P \leq 0.05$, (NS) not significant.

was 3.75% by using the commercial strain of *Aspergillus niger* and centrifugation of the juice prior to fermentation. Using of factorial design showed the importance of interaction effect that the parameters had on the responses. Dates can be a promising material for production of citric acid.

References


