Lactic Acid Production by Lactobacillus bulgaricus from MRS Medium without and with Replacement of Glucose by Date and Carob Pod Powders

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Abstract

The main aim of this present study was the study of the possibility to the replacement of glucose by date powder or carob powder in MRS medium and the effect of this replacement on the kinetic profile of the growth of Lactobacillus bulgaricus. The replacement of 20 g.L⁻¹ of glucose per 20 g.L⁻¹ of carob powder causes an increase in the cell mass of Lactobacillus bulgaricus and a quantity of lactic acid 5 g.L⁻¹ close to that obtained on glucose 6.65 g.L⁻¹. The replacement of glucose by 20 g.L⁻¹ of date powder results in an extension of the latency phase, which indicates a difficulty in adapting Lactobacillus bulgaricus to the date components, but after this period of latency the cell density increases to a value of 1.07 close to that obtained on glucose 1.26. The amount of lactic acid obtained is very low at 3.55 g.L⁻¹ relative to the quantities produced on glucose and carob powder respectively 6.65 and 5 g.L⁻¹.

Keywords
Carob; Date; Fermentation; Lactic Acid; Lactobacillus bulgaricus; Powders

Introduction

The date palm Phoenix dactylifera L. belonging to the Arecaceae family represents an important economical and ecological culture for many countries of Middle East and North Africa [1-3]. There is an important genetic biodiversity of the date palm in the Maghreb region (Algeria, Tunisia and Morocco) with more than 1000 varieties [4]. Algeria is a date producer country with an annual production of more than 5000000 t. The number of date palms in Algeria is estimated at more than ten million [5], the Deglet Nour variety is in fir place (46.23%) followed by Deglet Beidha (32.9%) [6], other varieties represents 30% of the national production, known as common are less quality. Theses varieties are very rich in nutrients elements (carbohydrates, vitamins, ash, and minerals salts). Date palm cultivars are divided to three main types according to their fruit moisture content as soft, semi-dry and dry cultivars [7]. Dates contain a high percentage of sugars reaching 88% in some varieties [8] and mineral salts [9] but low in fat and virtually free from Cholesterol and sodium. The dates are exploited as fermentation medium for various metabolites production: yea bread, ethanol and citric acid [10].

Carob tree (Ceratonia siliqua L.) has an economic, environmental and ornamental importance in Algeria.
for reforestation of arid and degraded areas [11, 12]. The pulp and the seeds are used as a raw material in food industry (live ock, biscuits), biotechnological industry, cosmetics and pharmacological industry (drug delivery) [13, 14]. The fruits have 19-92% of total dry matter and 62-67% total soluble solids, which characterized by high soluble sugars as 7-10% glucose, 10-12% fructose and 34-42% sucrose [15]. The carob is rich in fiber, calcium, antioxidants and phenolic compounds from 2 to 20% D.M. and poor in fatty acids 0.4 to 0.8% and proteins (2.7 to 7.6%) [14, 16, 17]. The phenolic compounds can be used as antioxidant additive [18-20]. The carob extract can be used for production of intereing products by fermentation [21-23]. The objective of this study was the determination of the kinetic profile of the growth of Lactobacillus bulgaricus from MRS medium without and with replacement of 20 (g.L-1) of glucose by 20 (g.L-1) of date or carob pods powders for production of lactic acid.

### Material and Methods

#### Vegetable Material

The carob (Ceratonia siliqua L.) used in current experiments was harvested in the month of June 2014 from the region of ELBORDJ (Mascara, Algeria) and the date (Phoenix dactylifera L.) was a variety half soft, known as Hmira badly exploited, collected in the month of June 2014 and cultivated in the area of Bechar (South-West of Algeria). The choice of these varieties is justified by its availability and important nutritive value, especially the one of reducing fermentable sugars such as glucose and fructose.

#### Preparation of Date and Carob Powders

For the preparation of the date and carob powders, theses fruits have been washed in order to eliminate sand and dust, and then cut into the small particles (1-3 cm). Vegetable material were dried at 80-90°C and the drying process was continued until the pulp moisture content did not decrease significantly with increasing drying time and/or the colour change is not visible to the naked eye (Figure 1). The moisture content thus obtained was considered as equilibrium moisture content [24, 25].

### Fermentation Conditions

The Lactobacillus bulgaricus rain was supplied by the Orolait of Mascara (Algeria). It was maintained on MRS medium in the presence of 10% glycerol and preserved at -20°C. The MRS medium used in culture contained the following components (in g.L-1): 10 Soya peptone, 10 beef extract, 5 yea extract, 2 K2HPO4, 5 NaCH3CO2, 3 H2O, 2 triammonium citrate, 0.2 MgSO4·7 H2O, 0.05 MnSO4·H2O, 20 glucose and 1ml Tween 80. The reactivation phase of rain was obtained after two successive transplantations at 42°C during 2 hours on liquid MRS broth [26]. All fermentations were carried out in a 2 L jar bioreactor (Applikon Biocontroller ADI1030) with an initial volume of 1.5 L. The inocula were incubated at 42°C for 12 h at 200 rpm before their transfer to the bioreactor in a 10%. The culture pH was maintained at 6.25 by automatic addition of NH4OH solution during time of the fermentations and the culture was sterilized at 108°C for 15 min. The samples were withdrawn at desired intervals and frozen for further analysis. Three fermentations of Lactobacillus bulgaricus were carried on MRS medium containing 20 (g.L-1) of glucose, MRS with 20 (g.L-1) of carob powder and MRS with 20 (g.L-1) of date powder.

### Analytical Methods

The biomass was determined by measurement of the optical density (OD) at 600 nm by a spectrophotometer HITACHI 4-2000. Culture samples were centrifuged (13200g at 4°C for 5 min), diluted and filtered. Residual glucose and lactic acid concentrations were determined by Multi parameter Medical Analyzer. The enzymatic kit used for the lactic acid dosages was the PAP Ref-61 192 and for the glucose dosages was the Elitech diagnosis ref - GPSL-0500.
Kinetic Parameters

The various analysis carried out allowed the following time evolution of the component concentrations present in the culture medium. From these raw data it was possible to calculate the fermentation kinetic parameters in the batch culture by the calculation of the specific rate of growth $\mu$ in h$^{-1}$, of sugars consumption $Q_S$ in g.g$^{-1}$.h$^{-1}$ and of lactic acids production $Q_{l.a}$ in g.g$^{-1}$.h$^{-1}$ [27].

$$\frac{dX}{dt} = \mu X$$
$$Q_S = \frac{dS}{dt}$$
$$Q_{l.a} = \frac{dP}{dt}$$

The maximal specific growth rate ($\mu_{max}$) was determined from the slopes of the plotted linear curve: $\ln X/X_0 = f(t)$. The productivity was defined as the amount of lactic acid produced per liter per hour. Yields of fermentation products (lactic acid (Y$_{l.a}$/S) and biomass (Y$_X$/S) were calculated from the slope of the raight line obtained by plotting the increasing amount of product (P-P0), and biomass (X-X0) as a function of sugar consumption, respectively. Yield of biomass (Y$_X$/S) is expressed in g X/g glucose; yield of fermentation products (lactic acid (Y$_{l.a}$/S) is expressed in g acid/g glucose [27].

Results and Discussion

The kinetic study of the fermentation of *Lactobacillus bulgaricus* on the MRS medium supplemented by 20 (g.L$^{-1}$) of glucose is characterized by an initial optical density of 0.33 at the beginning of the fermentation. This cell mass increases during the culture and reaches a final optical density of 1.59 after 8 hours of fermentation and abilizes at this value throughout the fermentation period.

The fermentation characterized by the subitation of 20 (g.L$^{-1}$) of glucose by 20 (g.L$^{-1}$) of carob powder is demonstrated by an initial optical density of 0.46. After 16 hours of fermentation, this density increases and reaches an OD of 1.9 indicating the use of the sugars present in the carob for cell growth. The absence of a latent phase indicates the perfect adaptation of *Lactobacillus bulgaricus* to MRS supplemented by carob powder. On the other hand, the replacement of glucose by 20 (g.L$^{-1}$) of date powder causes an extension of the latency period where cell division and growth begins only after 24 hours of growth and the maximum optical density obtained is 1.07 much lower compared to the other two fermentations (Figure 2, Table 1).

**Figure 2:** Evolution of Optical Density and Specific Rate of Growth $\mu$ in h$^{-1}$ during Batch Fermentations
The maximum specific rate $\mu_{\text{max}}$ (in h$^{-1}$) obtained for the three fermentations on glucose; carob powder and date powder are respectively 1.02, 0.37 and 0.24 h$^{-1}$. The low amount of sugar present in 20 g carob powder (2.35 g.L$^{-1}$) and date powder (2.85 g.L$^{-1}$) explains these low rates compared to 20 (g.L$^{-1}$) of glucose (Figure 3, Table 1). The quantity of sugars in the two natural products carob and date is 10-fold low compared to glucose which explains the maximum specific rate of consumption of sugars ($Q_{\text{Gluc max}}$ in g.g$^{-1}$.h$^{-1}$) which is 10-fold more on glucose (2.95) with respect to carob (0.2) and date (0.23).

The amount of lactic acid obtained on the MRS medium supplemented by 20 (g.L$^{-1}$) of carob powder is similar to that obtained on the MRS medium supplemented by 20 (g.L$^{-1}$) of glucose respectively 9 and 10 (g.L$^{-1}$) whereas the quantity obtained on MRS enriched with 20 (g.L$^{-1}$) of date powder is slightly less than 7 (g.L$^{-1}$) (Figure 4, Table 1).

This variation is reflected in the productivity of lactic acid, where the cultivation on MRS with glucose produces a productivity of (0.96 g.L$^{-1}$.h$^{-1}$) while the productivity obtained on MRS with carob powder is 0.75 and MRS with date powder is much less than 0.2 (Figure 5, Table 1).

The yields of conversion of the sugars into biomass are 0.09 on MRS supplemented with 20 (g.L$^{-1}$) of glucose, 0.86 (g.g$^{-1}$) on the substitution of glucose by carob powder and 1.27 when the glucose is replaced by 20 (g.L$^{-1}$) of date powder. The calculation of yields indicates that all sugars consumed during MRS fermentations auditioned by carob powder or dates powders are used for the growth and production of lactic acid (Table 1).

**Conclusion**

The bioconversion of agricultural by-products mainly the ones rich in fermentable sugars has an economic and rategic intere. The carob and date powders were very rich in carbohydrates which made them a substrate of choice for the development of high value added products. In producing countries, carob pods have traditionally been used as animal and human food and currently the main use is the seed for gum extraction, carob bean gum (CBG) or locu bean gum (LBG). The carob powder is being acclaimed as an ingredient with a marked nutritional value due to its high levels of dietary fiber (preventative role again heart disease) and phenol compounds (antioxidant activity). Regarding the high sugar content in date and carob pod, there have been some uudies on the production

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MRS with 20 (g.L-1) glucose</th>
<th>MRS with 20 (g.L-1) Carob powders</th>
<th>MRS with 20 (g.L-1) Date powders</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD $i.$</td>
<td>0.33 ± 0.03</td>
<td>0.46 ± 0.04</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>$f.$</td>
<td>1.59 ± 0.04</td>
<td>1.90 ± 0.03</td>
<td>1.45 ± 0.04</td>
</tr>
<tr>
<td>$P.$</td>
<td>1.26</td>
<td>1.44</td>
<td>1.07</td>
</tr>
<tr>
<td>Residual $i.$</td>
<td>19.7 ± 0.3</td>
<td>2.35 ± 0.2</td>
<td>2.85 ± 0.4</td>
</tr>
<tr>
<td>glucose $f.$</td>
<td>5.90 ± 0.2</td>
<td>0.68 ± 0.1</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>(g.L$^{-1}$)</td>
<td>C.</td>
<td>13.8</td>
<td>1.67</td>
</tr>
<tr>
<td>Lactic $i.$</td>
<td>3.35 ± 0.1</td>
<td>4 ± 0.2</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>acid $f.$</td>
<td>10 ± 0.3</td>
<td>9 ± 0.2</td>
<td>7.05 ± 0.1</td>
</tr>
<tr>
<td>(g.L$^{-1}$)</td>
<td>$\mu_{\text{max}}$ (h$^{-1}$)</td>
<td>1.02</td>
<td>0.37</td>
</tr>
<tr>
<td>$Q_{\text{Gluc max}}$ (g.g$^{-1}$.h$^{-1}$)</td>
<td>2.95</td>
<td>0.20</td>
<td>0.23</td>
</tr>
<tr>
<td>$Q_{\text{L.a max}}$ (g.g$^{-1}$.h$^{-1}$)</td>
<td>0.74</td>
<td>0.82</td>
<td>0.65</td>
</tr>
<tr>
<td>Productivity (g.L$^{-1}$.h$^{-1}$)</td>
<td>0.96</td>
<td>0.75</td>
<td>0.2</td>
</tr>
<tr>
<td>$Y_{x/s}$ (g.g$^{-1}$)</td>
<td>0.09</td>
<td>0.86</td>
<td>0.38</td>
</tr>
<tr>
<td>$Y_{L.a/s}$ (g.g$^{-1}$)</td>
<td>0.48</td>
<td>2.99</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Table 1: Kinetics Profile of Batch Fermentations ($i$: initial, $f$: final, $P$: quantity produced, $C$: quantity consumed). Values Presented are the Means of Triplicate Analysis
Figure 3: Evolution of Residual Sugars and Specific Rate of Sugars Consumption (\(Q_{\text{gluc}} \text{ in g g}^{-1} \cdot \text{h}^{-1}\)) during Batch Fermentations

Figure 4: Evolution of Lactic Acid and Specific Rate of Lactic Acid Production (\(Q_{\text{la}} \text{ in g g}^{-1} \cdot \text{h}^{-1}\)) during Batch Fermentations

Figure 5: Productivity of Lactic Acid during Batch Fermentations
of the value added product. Lactic acid can be one of these value added products due to its current and future potentials. The main aim of this work was study of the possibility to the replacement of glucose by date powder or carob powder in MRS medium and the effect of this replacement on the kinetic profile of the growth of \textit{Lactobacillus bulgaricus}. The replacement of 20 g.L\textsuperscript{-1} of glucose per 20 g.L\textsuperscript{-1} of carob powder causes an increase in the cell mass of \textit{Lactobacillus bulgaricus} and a quantity of lactic acid 5 g.L\textsuperscript{-1}. The replacement of glucose by 20 g.L\textsuperscript{-1} of date powder results in an extension of the latency phase, which indicates a difficulty in adapting \textit{Lactobacillus bulgaricus} to the date components, but after this period of latency the cell density increases to a value of 1.07 close to that obtained on glucose 1.26. The amount of lactic acid obtained is very low at 3.55 g.L\textsuperscript{-1} relative to the quantities produced on glucose and carob powder respectively 6.65 and 5 g.L\textsuperscript{-1}.

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References


