DETERMINATION OF BIOTIN CONTENT IN BEET MOLASSES
BY Lactobacillus plantarum

Eva S. Lončar, Irena S. Došenović, Siniša L. Markov, Radomir V. Malbaša and Ljiljana A. Kolarov

D-biotin content in beet molasses was determined by microbiological method using Lactobacillus plantarum, based on the comparison of the growth of this microorganism in molasses solutions with those in standard solutions of biotin. Incubation of the microorganism was performed on original Vitamin Biotin Testbouillon and laboratory prepared liquid culture medias. The amount of “real” biotin in molasses is low. The results depend upon the sample and volume of molasses solutions. Biotin contents obtained on both liquid media are close.

KEYWORDS: Biotin; molasses; Lactobacillus plantarum; biotin determination

INTRODUCTION

Molasses is the basic raw material for a lot of microbiological processes (1-3). For these processes it is very important that the molasses contains, besides carbon and nitrogen source, as many vitamins as possible, which are essential for important microorganisms such as Saccharomyces cerevisiae (4), Lactobacillus sp. and Micrococcus glutamicus (5). For yeast and alcohol and lactic and glutamic acid production a very important molasses parameter is biotin content. The total biotin activity of molasses towards yeast consists of the sum of the activities of biotin, desthiobiotin, oxybiotin, biocytin and possibly some other factors. On the other hand, the growth of lactobacilli such as Lactobacillus arabinosus, Lactobacillus casei and others is stimulated only by D-biotin (6).

Many microorganisms have been applied to the development of microbiological assay for biotin in various materials, e.g. Lactobacillus plantarum, Lactobacillus casei, Saccharomyces cerevisiae, Ochromonas dani, Neurospora crassa, Rhizobium trifolii, Escherichia coli 162, etc. (7).
The method with *Lactobacillus plantarum* is the most specific for determining D-biotin and its isomer form, biotin-D-sulfoxide (8,9), and biotin in various samples such as food material (10-13), human biological fluids (14) and lactose and beet molasses (9).

The purpose of the experiments is to determine biotin content in beet molasses samples with *Lactobacillus plantarum*. In the same samples dry matter, sucrose, purity, and pH value were also determined.

**EXPERIMENTAL**

**Materials**

Samples of molasses (1 and 2) were taken directly from production from two sugar refineries. They were ten-day samples of molasses composed of average daily samples. Every 4h samples of 1kg were taken from the production. After 24h these six samples were mixed and an average daily molasses sample was thus obtained.

Biotin for the preparation of standard solutions for the determination of biotin in molasses was used in the form of D(+)-biotin (C_{10}H_{16}N_{2}O_{3}S, M=244.31g/mol). It was “for biochemistry” quality (E. Merck, Darmstadt, Germany).

Test microorganism was *Lactobacillus plantarum* (ATCC 8014) from collection E. Merck, Darmstadt, Germany.

Agar culture media was Difco *Lactobacilli* agar AOAC (Difco, Detroit, USA).

Liquid culture media were Vitamin Biotin Testbouillon, VBT (E. Merck, Darmstadt, Germany) and laboratory prepared media, LP (15).

**Methods**

Dry matter was determined by refractometry with Schmidt-Haensch automatic precise refractometer type DUR-s (precision of determination 0.02% dry matter) (16,17).

Sucrose was determined polarimetrically with a Schmidt-Haensch saccharometer IV (reading precision 0.01%) (16,17).

Purity of molasses was calculated as in (18), i.e. ([wt sucrose/wt dry matter]x100).

pH value of molasses was measured on a pH-meter.

Biotin content was determined by the microbiological method with *Lactobacillus plantarum* according to the Methods for the Microbiological Analysis of Selected Nutrients-parts 300:Microbiological Methods and 310:Biotin (19). Standard solutions of biotin were 0.05; 0.1; 0.14 and 0.18 ng/mL. Molasses solution was prepared from 5 g of molasses in accordance with the procedure described in part 310Df (19).

Working inoculum was 0.1 mL solution of 0.1 g dry matter cell of *Lactobacillus plantarum*/10 mL sterile saline. Incubation was 22 h in a constant temperature bath at 37°C. One set solutions of biotin standard and molasses were 1, 2, 3 and 4 mL. For each level and volume of standard solutions and molasses solutions test tubes were prepared in triplicate and transmittance values, %T averaged (Spectrofotometer, Carl Ziss, Jena, Germany). Biotin content of the test molasses was calculated using the following equation:

\[
\text{Biotin content (μg/g molasses)} = \frac{(500xB)}{(MxV)}
\]

B-biotin content from standard curve, μg/mL; M-weight of molasses, g; V-volume of molasses solution, mL.

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RESULTS AND DISCUSSION

The results of dry matter and sucrose content, purity and pH for tested samples of molasses are shown in Table 1. The presented results show that the molasses was of very high quality (20).

Table 1. The results of dry matter and sucrose content, purity and pH in molasses samples

<table>
<thead>
<tr>
<th>Molasses</th>
<th>Dry matter (%)</th>
<th>Sucrose (%)</th>
<th>Purity (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83.00</td>
<td>47.70</td>
<td>57.47</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>85.20</td>
<td>48.00</td>
<td>56.34</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Microbiological assay for the determination biotin is based on the comparison of the growth of test organism *Lactobacillus plantarum* in the assay solution of molasses with that in a standard solution of biotin. Standard concentration-response curve was prepared by plotting the average %T readings for each level of standard solution used against amount of reference standard contained in respective tubes. Parallel straight lines were obtained as shown in Figs. 1 and 2. Correlation coefficients from linear regression analysis of experimental %T values varied from 0.9869 to 0.9989. The amount of biotin per milliliter for each tube at each level of sample assay solution is determined by standard curve interpolation. The results of biotin content in the tested molasses samples on two liquid culture media are shown in Table 2.

![Fig. 1. Plots of %T against biotin content in the standard and molasses solutions on Vitamin Biotin Testbouillon liquid media](image-url)
Table 2. Biotin content in samples of molasses on different liquid culture medias

<table>
<thead>
<tr>
<th>Molasses</th>
<th>μg biotin / g molasses</th>
<th>VBT media</th>
<th>LP media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mL 2 mL 3 mL 4 mL</td>
<td>1 mL 2 mL 3 mL 4 mL</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0.0185 0.0175 0.0147 0.0146</td>
<td>0.0187 0.0169 0.0140 0.0145</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.0228 0.0221 0.0180 0.0180</td>
<td>0.0210 0.0201 0.0180 0.0180</td>
</tr>
</tbody>
</table>

It is evident from the data in Table 2 that, in general, the content of “real” biotin, i.e. D-biotin in the molasses is low. Although the purity of molasses 1 and 2 is very similar (Table 1), the biotin content in molasses 2 is higher (21). Biotin content depends upon volume of assay solution of molasses and decreases with increasing the volume of molasses solution on both liquid culture media. Namely, in the solutions of 3 mL and 4 mL biotin content is lower. The colour of these solutions is darker and this colour interfere with turbidimetric measurements.

Results of experiments leading to the determination of D-biotin in molasses comply with the expectations. Low biotin level was found with *Lactobacillus plantarum* as compared with biotin level determined with *Saccharomyces cerevisiae* (22). This means that total biotin activity of molasses is higher than D-biotin content. *Lactobacillus plantarum* is used to identify and determine only biotin and biotin sulfoxide, whereas the use of *Saccharomyces cerevisiae* gives the total biotin activity, i.e. the sum of the activities of
biotin, dethiobiotin, biotin sulphoxide, oxybiotin, biocytin, and possibly some other factors (6,9).

Laboratory prepared liquid media, LP (15), is a good alternative to the original Vitamin Biotin Testbouillon liquid media, VBT. Substitution of VBT media (Fig. 1) with LP media (Fig. 2) resulted in decreasing the transmittance and the slopes of straight lines, but biotin contents on these media were close (Table 2).

CONCLUSIONS

Microbiological method with *Lactobacillus plantarum* enables the determination D-biotin content in molasses.

In the investigated molasses content of the “real” biotin, i.e. D-biotin which exhibits growth stimulation to lactobacilli, is low.

Laboratory prepared liquid culture media for incubation of *Lactobacillus plantarum* can be used in the analysis instead of Vitamin Biotin Testbouillon liquid media.

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REFERENCES


ОДРЕЂИВАЊЕ САДРЖАЈА БИОТИНА У МЕЛАСИ ШЕЋЕРНЕ РЕПЕ ПОМОЋУ Lactobacillus plantarum

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Садржај D-биотина у меласама шећерне репе је одређен микробиолошком методом помоћу Lactobacillus plantarum на основу поређења раста овог микроорганизма у растворима меласа са растом у стандардним растворима биотина. Инкубација микроорганизма је изведена на оригиналној Vitamin Biotin Testbouillon и лабораторијски припремљеној течној подложи. Количина "правог" биотина у меласама је мала. Резултати зависе од узорка и запремине раствора меласе. Блиске вредности за садржај биотина су добијене на обе течне подлоге.

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