IMMOBILIZATION OF A LACTOSE-BINDING LECTIN FOR LACTOSE REMOVAL FROM MILK

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RESUMO – Este trabalho apresenta a extração, purificação parcial e caracterização de uma lêctina de sementes de Brosimum gaudichaudii. A lêctina extraída foi imobilizada em polianilina ativada com glutaraldeído (PANIG) e utilizada para remoção de lactose. O melhor rendimento de extração foi encontrado utilizando-se tampão glicina 0.1 mol L⁻¹ pH 9.0, sendo observado 505.2 unidades de hemaglutinina por milígrama de proteína. O extrato bruto foi parcialmente purificado utilizando-se cromatografia por gel filtração (Sephadex G75), e a lêctina parcialmente purificada foi caracterizada como lêctina ligante de lactose. Essa lêctina foi imobilizada em PANIG e o complexo PANIG-lêctina foi eficiente em remover 47,1% da lactose presente em leite de vaca. Estes resultados evidenciaram que o sistema PANIG-lêctina pode ser uma alternativa promissora para a produção de produtos com baixa concentração ou sem lactose.

PALAVRAS-CHAVE: Brosimum gaudichaudii, produtos sem lactose, imobilização.

ABSTRACT – In this work, a lectin from seed of Brosimum gaudichaudii was extracted, partially purified, characterized and immobilized onto glutaraldehyde polyaniline derivative (PANIG) for lactose removal. The best extractor tested was found as 0.1 mol L⁻¹ glycine buffer pH 9.0, being reached 505.2 hemaglutinating units per milligram of protein. This crude extract was partially purified through gel filtration chromatography, and the partially purified lectin was characterized as a lactose-binding lectin. This lectin was immobilized onto PANIG and the complex PANIG-lectin presented 47.1% of lactose removal from milk. These results evidenced that PANIG-lectin can be a promising alternative to produce lactose low-concentration or lactose-free dairy products.

KEYWORDS: Brosimum gaudichaudii; lactose-free products; immobilization.

1. INTRODUCTION

The use of lactose as ingredient for food products is limited because of their low solubility, low sweetness and unattractive-ness by people with lactose intolerance disease. The segment of the world population showing permanent or temporary lactose intolerance is quite significant. Symptoms of lactose intolerance can include nausea, vomiting, and diarrhea, with the severity of symptoms dependent on the level of lactose intolerance (Sudsa-ard et al., 2014; Vieira et al., 2013).

In addition, some individuals suffer from the genetic metabolic disorder galactosemia and do not tolerate lactose and additionally galactose. In this case, lactose-free dairy products, in which lactose is enzymatically hydrolyzed to glucose and galactose and from which the latter is not subsequently removed, are not suitable for patients with galactosemia (Morlock et al., 2014). For this
reason, considering that milk products are widely consumed and present a high nutritional value, technological alternatives have been sought to overcome this dilemma. Among the emerging alternatives, the use of systems that remove lactose from milk are quite promising.

Considering that lectins are non-immunogenic proteins which selectively and reversibly bind stereospecific carbohydrates and derivatives, the objective of this work was to extract a lactose-binding lectin from seed of *Brosimum gaudichaudi* and immobilize it onto glutaraldehyde-modified polyaniline for use as lactose removal system.

2. MATERIAL AND METHODS

2.1 Lectin extraction

The extraction of lectin from seeds of *B. gaudichaudi* was carried out using different solvents (0.1 mol L\(^{-1}\) glycine buffer pH 2.6 and 9.0; 0.15 mol L\(^{-1}\) saline solution). One gram of flour was mixed with 10 mL of extractor, incubated at 4 °C for 30 min. Then, the crude extract was centrifuged for 5 min at 8000 rpm and used for hemagglutinating assays. Total protein was determined according to methodology described by Bradford (1976), using bovine serum albumin as standard.

2.2 Hemaglutinating activity

Hemagglutination assays were carried out as described by Moreira and Perrone (1977), using rabbit red blood cells. For the hemagglutination test, 200 μL of a 2 % (v/v) erythrocyte suspension were added to an equal volume of a lectin solution and the mixture incubated at 37° C for 30 min, followed by incubation at room temperature for 30 min. The hemagglutination title was defined as the minimal amount of protein able to induce visible erythrocyte agglutination and assumed as one hemagglutinating unit (HU).

2.3 Partial purification and characterization

The partial purification was carried out by exclusion size chromatography (Sephadex G-75) using Äkta Prime Plus system, with detection at 280 nm. The peak that showed hemagglutinating activity was pooled and used for the immobilization assays. Carbohydrate-binding specificity of the *B. gaudichaudii* lectin was assessed by the ability of different sugars to inhibit agglutination of rabbit erythrocytes, measured by preparing two-fold serial dilutions of the sugar solutions (1.0 mol L\(^{-1}\) initial concentration) in 0.15 mol L\(^{-1}\) saline solution.

2.4 Lectin immobilization onto polyaniline

Polyaniline (PANI) was synthesized and activated with glutaraldehyde (PANIG) as described by Fernandes et al., (2003). The resulting PANIG was exhaustively washed with water to assure the complete removal of glutaraldehyde. Then, PANIG was dried and stored at room temperature until use in the immobilization experiments. The optimum immobilization conditions were achieved using a 23 Central Composite Rotatable Design (CCRD). In addition, a central point with two replicates was also included for statistical evaluation (Table 2).

2.5 Lactose removal assays

In order to determine the stoichiometry of lactose-binding, assays were carried out by incubating 500 μL of a lactose solution with 25 mg of PANIG-lectin, at room temperature for 1h under stirring. Then, the complex was removed from reaction by centrifugation at 10000 rpm for 5 min and lactose remaining in the supernatant was determined using the method described by Bernfeld (1955), using 3,5-dinitrosalicylic acid (ADNS). The percentage of lactose removal was determined by the
difference between the total and the residual lactose content. In addition, the PANIG-lectin was tested for removal of lactose using defatted milk.

2.5 Statistical analysis

All tests were conducted according to a completely randomized model and the results expressed as means ± standard deviation. Results from CCRD were analyzed using the software Statistica 6.0 (Statsoft Inc., Tulsa, USA, 1997).

3. RESULTS AND DISCUSSION

The extraction optimization of *B. gaudichaudii* lectin was tested using different solvents. As can be seen in Table 1, high amounts of protein were extracted using saline or glycine buffer pH 9.0. However, extraction with glycine buffer pH 9.0 resulted in two-fold higher extraction compared to saline solution, providing the highest specific activity (505 HU mg⁻¹ protein). Considering these results, the lectin extracted using glycine buffer pH 9.0 was chose for characterization, partial purification and immobilization tests.

Table 1 – Protein content and hemaglutinating activity for *B. gaudichaudii* lectin extracted in different solvents.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Title (HU)</th>
<th>Protein (mg g⁻¹)</th>
<th>Specific Activity (HU mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine buffer 0.1 mol L⁻¹ pH 2.6</td>
<td>2560</td>
<td>25.42</td>
<td>100.7</td>
</tr>
<tr>
<td>Saline solution 0.15 mol L⁻¹</td>
<td>10280</td>
<td>39.58</td>
<td>259.7</td>
</tr>
<tr>
<td>Glycine buffer 0.1 mol L⁻¹ pH 9.0</td>
<td>20560</td>
<td>40.70</td>
<td>505.2</td>
</tr>
</tbody>
</table>

The sugar specificity of *B. gaudichaudii* lectin was tested against glucose, galactose, lactose and N-acetylglucosamine. Among the tested sugars, only lactose was able to completely inhibit the hemagglutinating activity at concentration of 0.5 mmol L⁻¹. This result constitutes an important finding that allows propose biotechnological applications for *B. gaudichaudii* lectin, such as its use for lactose removal in milk.

The *B. gaudichaudii* lectin was partially purified through size exclusion chromatography (Figure 1). The first peak observed in the chromatogram (52-65min) presented hemagglutinating activity, reaching a specific activity of 2319 HU mg⁻¹ protein. This lectin-enriched fraction was used for immobilization assays. The exclusion chromatography was very efficient, resulting in about 4.6-fold purification in a single step.
In this work, a $2^3$ Central Composite Rotatable Design (CCRD) was used in order to optimize the immobilization of *B. gaudichaudii* lectin onto PANIG. The efficiency of immobilization was evaluated by analyzing the percentage of lactose removal from a standard solution (Table 2).

Table 2 – Results of lactose removal for the lectin immobilization onto PANIG.

<table>
<thead>
<tr>
<th>PANIG (mg)</th>
<th>Time (min)</th>
<th>Lactose removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5</td>
<td>18.2</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>21.0</td>
</tr>
<tr>
<td>10</td>
<td>35</td>
<td>17.2</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>35.8</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>24.9</td>
</tr>
<tr>
<td>20</td>
<td>35</td>
<td>29.1</td>
</tr>
<tr>
<td>30</td>
<td>5</td>
<td>30.3</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>30.5</td>
</tr>
<tr>
<td>30</td>
<td>35</td>
<td>30.1</td>
</tr>
</tbody>
</table>

Results from multivariate analysis (ANOVA) evidenced that the linear term for PANIG concentration positively affected the response while the quadratic term showed a negative effect on the response ($r^2=0.80$). According to the mathematical prediction of desirability, the best condition of immobilization would be 25 mg of PANIG and 5 min of immobilization time. Using this prediction, the experimental data showed a lactose removal of 46.3% ($\pm 0.92$).

Furthermore, tests with defatted bovine milk showed that the PANIG-lectin complex is a very promising material. Tests conducted by reacting 25 mg of PANIG-lectin with 500 μL of milk containing 650 μg of lactose resulted in a removal of 47% lactose (305.5 μg).
4. CONCLUSION

The lectin extracted from seeds of *Brosimum gaudichaudii* was classified as a lactose-binding lectin. The best immobilization results were achieved when lectin was left to react with 25 mg PANIG for 5 min, at room temperature. The PANIG-lectin complex was able to remove 46.3% of lactose using as start material defatted milk. These results allow suggesting that PANIG-lectin is a promising material to be used in processes to reduce or eliminate the lactose in milk and dairy products.

5. ACKNOWLEDGEMENTS

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6. REFERÊNCIAS BIBLIOGRÁFICAS


