BIOACTIVE EDIBLE COATING FOR SHELF LIFE IMPROVEMENT OF ORGANIC STRAWBERRIES

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ABSTRACT – Organic foods have great importance in environment sustainability and human nutrition. However, they are commercialized with greatly reduced shelf life, especially fruits and vegetables. Considerable amounts of vegetables are lost due to their rapid deterioration by microorganisms such as fungi and bacteria. In this work cell wall degrading enzymes (CWDE) produced by Trichoderma asperellum (T00) were immobilized in cashew gum polysaccharides (CGP) in order to use this material as edible coating of fruits, aiming to increase shelf life of strawberries by inhibiting fungal growth. Results showed that optimum conditions for CWDE immobilization were 20 min reaction and 1 mM of sodium periodate concentration. The CGP-CWDE complex has maintained about 70% of initial activity after 23 days of storage at room temperature. Additionally, CGP-CWDE was effective to inhibit the growth of Penicillium sp., Aspergillus fumigatus and Sclerotinia sclerotiorum.

KEYWORDS: Cashew gum, N-Acetylglucosaminidase, chitinase, shelf life.

1. INTRODUCTION

Organic foods have great importance in environment sustainability and human nutrition. These are the main reason to explain the increase in the interest of consumers and marketers to this kind of food. The concern with environmental effects of pesticides, genetically-modified organisms and food safety strongly reinforce this tendency. Despite of their importance, these foods are commercialized with greatly reduced shelf life, especially fruits and vegetables. In general, large amounts of vegetables are lost due to their rapid deterioration by microorganisms such as fungi and bacteria (Rembialkowska, 2007). In this sense, several studies have been directed to the development of bioactive packaging, designed to improve both the sensory qualities as well as the shelf life by inhibiting contamination by microorganisms. Bioactive packs generally consist of a polymer in which was introduced an agent capable of promoting the conservation and sanitization of the food during storage (López de Lancey et al., 2012).

Several polysaccharides have been studied for the production of polymeric matrixes with applications in many areas of biotechnology. These polysaccharides include alginate (Song et al., 2011), chitosan (Elsabee; Abdou, 2013), pectin (Espitia et al., 2014) and more recently, cashew gum polysaccharides (CGP) (Silva et al., 2012). CGP is a complex branched heteropolysaccharide produced as exudate after mechanical or biological injuries in the stem of Anacardium occidentale tree. This material has been used for production of polymeric matrixes in several applications as
systems for release of larvicides and encapsulation of essential oils (Oliveira et al., 2014), in the development of materials with potential biomedical applications (Moreira et al., 2015; Silva et al., 2016) and as matrixes for enzymes immobilization (Silva et al., 2010; Silva et al., 2012).

In a previous study, cell wall degrading enzymes (CWDE) produced by T. asperellum (T00) in the presence of crude chitin were immobilized in blended films containing CGP and polyvinyl alcohol (PVA). These films were effective for food preservation avoiding fungi proliferation. However, CGP based films were opaque, which limited its application as packaging due to the low consumer acceptance (Silva et al., 2012). Thus, the objective of this work was to immobilize CWDE produced by T. asperellum directly in CGP in order to use this powder as edible coating for fruits, aiming to increase the shelf life of strawberries by inhibiting fungal growth.

2. MATERIAL AND METHODS
2.1. Strain Origin and Culture Conditions.

T. asperellum (strain T00) was obtained from the culture collection of the Laboratório de Enzimologia at Universidade Federal de Goiás and maintained in potato-dextrose-agar medium (PDA), at room temperature (25 °C). The production of CWDE was carried out according to method described by Silva et al. (2011).

2.2. Cashew gum polysaccharide: origin and immobilization of the CWDE.

CGP was extracted according to methodology described by Silva et al (2010). Covalent immobilization of CWDE onto CGP was tested by adding 50 μL of CDWE solution (5 mg mL⁻¹) to 5 mg mL⁻¹ of CGP solution contain 400 μL sodium periodate solution and 1.55 mL of 50 mM sodium acetate buffer, pH 5.0. The CGP-CWDE complex was precipitated by adding 6 mL of ethanol, separated by centrifugation at 5000 g for 5 min, dried and stored at room temperature (25 °C). The optimum immobilization conditions were achieved using a 2³ Central Composite Rotatable Design (CCRD). The parameters and levels selected for this study sodium periodate concentration (1, 10 and 100 mmol L⁻¹) and immobilization time (20, 40 and 60 min). In addition, a central point (10 mmol L⁻¹ and 30 min), with two replicates was also included for statistical evaluation (Table 1).

2.3. Enzymatic assays

2.3.1. Activity of free and immobilized CWDE

N-Acetylglucosaminidase (NAGase) activity was determined by monitoring the rate of formation of p-nitrophenol from p-nitrophenol-β-N-acetylglucosamine, according to the modified method of Yabuki et al., (1986). The amount of released p-nitrophenol was determined at 405 nm. One unit of enzyme activity (U) was defined as the amount of enzyme that releases 1 μmol of p-nitrophenol min⁻¹ at 37 °C.

Chitinase activity was determined according to methodology of Molano et al., (1977), using colloidal chitin prepared as described by Berger and Reynolds (1958). Assays were performed incubating 250 μL of colloidal chitin and 250 μL of CWDE solution at 37 °C by 2 h, and the reducing sugar produced was determined by the method described by Miller (1959). One unit of enzyme (U) was defined as the amount of enzyme necessary to produce 1.0 μmol of reducing sugar in 2 h of reaction. Assays of immobilized CWDE were done similarly, except the CWDE solution was replaced by the CGP-CWDE complex. Blank reactions were performed in the presence of CGP activated by sodium periodate and in absence of enzymes.

2.4. Antifungal activity of immobilized CGP-CWDE

The fungal growth inhibition of the immobilized enzymes was determined against Penicillium sp., A. fumigatus and S. sclerotiorum in solid medium, in the presence of CGP-CWDE complex.
The antifungal activity was tested on strawberries samples. Strawberries were immersed in a solution of CGP-CWDE (3 mg mL\(^{-1}\)), left to dry at room temperature and immersed in a solution containing \(10^7\) spores mL\(^{-1}\) of *Penicilium sp.* Samples were maintained at room temperature (25 °C) for 4 days. Control assays were performed with samples that received only inoculums of microorganisms and CGP solution (3 mg/mL) followed by inoculum immersion.

3. RESULTS AND DISCUSSION

The immobilization of chitinolytic enzymes occurred by covalent linkages between polysaccharide and the glucosyl groups present in CWDE. Results obtained in experimental design are shown in Table 1.

<table>
<thead>
<tr>
<th>Variable level</th>
<th>Response (NAGase (U/mg))</th>
</tr>
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<tbody>
<tr>
<td>Run NaIO(_4) (mmol L(^{-1})) (X_1)</td>
<td>Time (min) (X_2)</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>1 1.0 (-1)</td>
<td>20 (-1)</td>
</tr>
<tr>
<td>2 1.0 (-1)</td>
<td>40 (0)</td>
</tr>
<tr>
<td>3 1.0 (-1)</td>
<td>60 (1)</td>
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<tr>
<td>4 10 (0)</td>
<td>20 (-1)</td>
</tr>
<tr>
<td>5 10 (0)</td>
<td>40 (0)</td>
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<tr>
<td>6 10 (0)</td>
<td>60 (1)</td>
</tr>
<tr>
<td>7 100 (1)</td>
<td>20 (-1)</td>
</tr>
<tr>
<td>8 100 (1)</td>
<td>40 (0)</td>
</tr>
<tr>
<td>9 100 (1)</td>
<td>60 (1)</td>
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</table>

As shown in Pareto chart (Figure 1), both reaction time and NaIO\(_4\) concentration negatively influenced the efficiency of NAGase immobilization. The optimum immobilization of NAGase occurred at conditions described in run 1 (20 min of reaction and activation with 1 mM of sodium periodate). The negative effect of NaIO\(_4\) concentration may be explained due to the oxidant effect of this compound on the structure of CGP and CWDE. Increasing the NaIO\(_4\) concentration may lead to degradation of CGP tridimensional network, compromising the structure of the support for CWDE immobilization. On the other hand, the contact of CWDE with the oxidant NaIO\(_4\) may result in oxidation of enzymes, which may compromise their activity. This can be the main reason to the negative effect of time reaction, since increasing time increases the contact among NaIO\(_4\) and others reaction components. On the conditions described at run 1, 0.3 units of chitinase were also immobilized.

Figure 1. Pareto chart and surface response for NAGase immobilization, respectively.
After immobilization, the CGP-CWDE complex was dried and stored at room temperature (25°C) for 23 days (Figure 2). Tests of NAGase activity showed that CGP-CWDE complex was very stable under this storage condition, maintaining 70% of the initial activity at the 23th day. Storage stability is an important characteristic of immobilized enzymes (Fernandes et al., 2013). In this case, the stability acquired after immobilization is important for applications in the food industry. The high stability of CGP-CWDE allows the use of this complex to restrain fungal growth in food packages, enhancing the shelf life of packaged foodstuffs.

**Figure 2.** Storage stability of NAGase immobilization.

In order to verify if CGP-CWDE complex was able to inhibit fungal growth, tests were conducted in the presence and absence of this complex. As can be seen in Figure 3, CGP-CWDE complex efficiently inhibited the growth of *Penicillium* sp., *Aspergillus fumigatus* and *Sclerotinia sclerotiorum*, which are fungus that frequently contaminate food packages.

**Figure 3.** Fungal growth inhibition by immobilized CGP-CWDE after 4 days of growth at 37 °C: A) – *Penicillium* sp.; B) – *Penicillium* sp. and CGP; C) – *Penicillium* sp. and CGP-CWDE; D) – *A. fumigatus*; E) – *A. fumigatus* and CGP; F) – *A. fumigatus* and CGP-CWDE; G) – *S. sclerotiorum*; H) – *S. sclerotiorum* and CGP and I) – *S. sclerotiorum* and CGP-CWDE.
Figure 4 shows the results obtained in tests using the CGP-CWDE complex to inhibit growth of *Penicillium sp.* on strawberries. As can be seen, after 3 days at storage at room temperature, the sample containing CGP-CWDE presented better appearance when compared with the samples of control. CGP-CWDE completely inhibited fungal growth on strawberries that received the complex previously to inoculum. Additionally, treated strawberries maintained the same initial color and were slightly shiny whereas untreated strawberries and those that received Penicillium inoculum presented clear signs of deterioration.

**Figure 4.** Fungal growth inhibition by immobilized CGP/CWDE after 4 days of storage at room temperature: A) – CGP; B) – CGP-CWDE; C) – CGP-CWDE and *Penicillium sp.*; D and E) – *Penicillium sp.*

4. CONCLUSIONS

These results suggest that CGP-CWDE has excellent applications in the food industry mainly as additive in the development of bioactive edible coatings. The efficient immobilization of NAGase
and chitinase were determinant to make CGP-CWDE effective in inhibiting the growth of fungi when tested in Penicillium sp., A. fumigatus and S. sclerotiorum. Tests with strawberries showed a potential and immediate application of CGP-CWDE complex.

5. ACKNOWLEDGEMENTS

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3. REFERENCES


