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Improvement of antioxidant and antimicrobial activity of soy isoflavones extracts bioconverted with β-glucosidase

Melhoria da atividade antioxidante e antimicrobiana de extratos de isoflavonas de soja bioconvertidos com β-glucosidase

DOI:10.34117/bjdv6n1-306

Recebimento dos originais: 30/11/2019
Aceitação para publicação: 27/01/2020

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ABSTRACT

Soybean is largely produced in Brazil being one of the main Brazilian Agricultural crop, achieving 223 million tons produced in 2017. The consumption of soy products increased in recent years due to the association with healthy benefits in oxidative stress and chronic diseases. The researches have been pointing that the isoflavones presenting in soy are the responsible compounds for alleviating these symptoms. The main purpose of this work were use of β-glucosidase enzyme to convert glucoside isoflavones to aglycone form in isoflavone soy extract, and then evaluate the antioxidant and antimicrobial activity against some pathogens. The isoflavones were obtained according to Aguiar (2004) with modifications. The soy flour (65 mesh) was defatted with hexane (1:10 w/v) and the extraction of isoflavones was performed using 80% aqueous methanol solution under 100 rpm

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stirring for 1 hour at room temperature. The mixture was then filtrated, and the supernatant recovered. The extract was used for the bioconversion of glycosyl isoflavones from soy into aglycones isoflavones using β-glucosidase enzyme. According to recent researches the aglycon form has greater absorption by organism and higher antioxidant activity than the glucoside form. According the results all antioxidant methods presented higher activity to bioconverted extract. The bioconverted extract (1060.73 mg Trolox equivalent) presented near 9 times superior antioxidant activity measured by DPPH method than raw extract (123.21 mg Trolox equivalent). The Total phenolic content presented similar behavior being higher to bioconverted extract than in raw extract. The antimicrobial activity of the isoflavones extracts raw and bioconverted against S. aureus, C. albicans and E. coli were higher than 1600 μg/mL in the range studied 0.78 to 1600 μg/mL.

Keywords: soybean, isoflavones, β-glucosidase.

RESUMO

A soja é amplamente produzida no Brasil, sendo uma das principais culturas agrícolas brasileiras, atingindo 223 milhões de toneladas produzidas em 2017. O consumo de produtos de soja aumentou nos últimos anos devido à associação com benefícios saudáveis ao estresse oxidativo e doenças crônicas. As pesquisas apontam que as isoflavonas presentes na soja são os compostos responsáveis por aliviar esses sintomas. O principal objetivo deste trabalho foi o uso da enzima β-glucosidase para converter isoflavonas de glucosídeo em forma de aglicona no extrato de soja de isoflavona e, em seguida, avaliar a atividade antioxidante e antimicrobiana contra alguns patógenos. As isoflavonas foram obtidas de acordo com Aguiar (2004) com modificações. A farinha de soja (malha 65) foi desengordurada com hexano (1:10 p / v) e a extração de isoflavonas foi realizada usando solução aquosa de metanol a 80% sob 100 rpm com agitação por 1 hora à temperatura ambiente. A mistura foi então filtrada e o sobrenadante recuperado. O extrato foi utilizado para a bioconversão de glicosil isoflavonas da soja em agliconas isoflavonas utilizando a enzima β-glucosidase. De acordo com pesquisas recentes, a forma aglicona tem maior absorção pelo organismo e maior atividade antioxidante que a forma glucosídica. De acordo com os resultados, todos os métodos antioxidantes apresentaram maior atividade ao extrato bioconvertido. O extrato bioconvertido (1060,73 mg equivalente a Trolox) apresentou atividade antioxidante quase 9 vezes superior medida pelo método DPPH do que o extrato bruto (123,21 mg equivalente a Trolox). O conteúdo fenólico total apresentou comportamento semelhante, sendo maior no extrato bioconvertido do que no extrato bruto. A atividade antimicrobiana dos extratos de isoflavonas brutas e bioconvertidas contra S. aureus, C. albicans e E. coli foi superior a 1600 μg / mL na faixa estudada de 0,78 a 1600 μg / mL.

Palavras-chave: soja, isoflavonas, β-glucosidase.

1 INTRODUCTION

Soybean is largely produced in Brazil being one of the main Brazilian Agricultural crop, achieving 223 million tons produced in 2017 (FAO, 2018). The consumption of soy products increased in recent years due to the association with healthy benefits in oxidative stress and chronic diseases (Eason et al., 2005). The researches have been pointing that the isoflavones presenting in soy are the responsible compounds for alleviating these symptoms. Isoflavones are diphenolic compounds of plants and they are categorized chemically by their functional groups as aglycons, glucosides, malonylglucosides and acetylglucosides. Isoflavone aglycones have been shown to

possess greater bioactivity and bioavailability than the other three forms, being readily absorbed in the small intestine (Chen et al., 2013; Lima & Ida, 2014).

B-glucosidase is an important enzyme that is capable to break β 1,4 linkages in various disaccharides, oligosaccharides, alkyl- and aryl-β-D-glucosides and is widely distributed in nature and is particularly common in plant seeds (Kudou, 1991; Mallek-Fakhfakh et al., 2017). This enzyme are involved in an important roles in a variety of fundamental biological processes, including the hydrolysis in glucosides of isoflavones (Matsuura and Obata, 1993).

In order to increase the aglycones amount in isoflavones soy extract, the β-glucosidase from *A. niger* LBA 02 was added and antioxidant and antimicrobial activity was mesuared and compared with raw extract.

2 MATERIAL AND METHODS

2.1 B-GLUCOSIDASE PRODUCTION

β-glucosidase from Aspergillus niger LBA 02 (strain deposited at the Culture Collection of Food Biochemistry Laboratory, University of Campinas, Campinas-SP, Brazil) was produced by semi solid fermentation. Briefly, the culture medium was composed of wheat bran and coffee peel (1:1) 100 g in saline solution conpousend by 0,1% of KN_2PO_4, 0,1% of $NaN0_3$ e 0,05% of yeast extract in distilled waterthen 10^7 spores per mL were added to the Erlenmeyer flask with the culture medium (10 g) and incubated at 30 °C for 7 days. The enzyme was extracted by the addition of an aliquot of 50 mL of distilled water was added to the Erlenmeyer flasks for the extraction procedure. The solution was maintained in agitation at 150 rpm for 20 minutes. After filtration, an ammonium sulfate solution (80% of saturation) was added to the enzyme extract and the mixture was stored at 3 ºC overnight. The suspension was centrifuged (10 minutes, 10.000 rpm), and the solid residue was resuspended in 0.05 M sodium phosphate buffer pH 7.0. The extract was freezed and used to bioconvert the soy isoflavones as described in item 2.3.

2.2 SOYBEAN GLYCOSYL ISOFLAVONES EXTRACTION

The isoflavones were obtained according to Aguiar (2004) with modifications. The soy flour (65 mesh) was defatted with hexane (1:10 w/v) and the extraction of isoflavones was performed using 80% aqueous methanol solution under 100 rpm stirring for 1 hour at room temperature. The mixture was then filtrated, and the supernatant recovered. The extract was used for the bioconversion of glycosyl isoflavones from soy into aglycones isoflavones using β-glucosidase enzyme.

2.3 ISOFLAVONES EXTRACT BIOCONVERSION WITH B-GLUCOSIDASE FROM *A. NIGER*

Falcon flasks containing 10 mL aliquots of isoflavones extract and 1 mL of β-glucosidase solution (0.1 mg mL^{-1}) (2.53 U/mL) in acetate buffer 0.1 M pH 4.5 were incubated at 50 °C, under

stirring for 24 hours. The reactions were stopped by freezing the Falcon flasks. The crude extract was used as control. The control and bioconverted extracts were used as samples to measure antioxidant and antimicrobial activity.

2.4 ANTIOXIDANT ACTIVITY

2.4.1 Total phenolics

The total phenolic content of the extracts were determined according to the Folin-Ciocalteu spectrophotometric method with some modifications. A calibration curve in the range of 16 to 300 µg/mL of gallic acid was prepared. From each calibration solution, samples and water (Blanch), aliquots of 50 µL was mixed with 800 µL of distilled water and 50 µL of Folin-Ciocalteu's phenol reagent and allowed to react for 3 min. Then, 100 µL Na_2CO_3 solution was added, and the mixture. was incubated during 2 hours in dark room at room temperature. After this period the absorbance at 760 nm was determined by spectrophotometer. The test was done in triplicate.

2.4.2 DPPH

The antioxidant activity of the extracts were determined using the DPPH free radical scavenging assay described by BOUGATEF (2009) with some modifications. Measurements were taken in triplicate. DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \left\{\frac{A0-A}{Ao}\right\}\times 100 \quad (1)$$

where A0 is the absorbance of negative control (0.004% DPPH solution) and A is the absorbance in presence of sample. The results were reported as Trolox equivalent.

2.4.3 FRAP

Experiments were done according to Benzie e Strain (1996) with modifications.

2.5 ANTIMICROBIAL ACTIVITY

To evaluate the antimicrobial activity, the method of broth microdilution (CLSI, 2008; CLSI, 2015) was used with modifications. The assay was performed in Mueller Hinton broth. The inoculums were adjusted to 75% transmittance at 660 nm, corresponding to $1.5x10^8$ UFC.mL^{-1}. The sample (8 mg. mL^{-1}) was prepared in absolute ethanol from 0.39 to 400 µg/mL in 96-well plates. The microplates were incubated at 35±2 ºC for 24h. A solution of resazurin (0.02%) was used for the determination of microbial growth, which was indicated visually by color changes (from blue to pink). The lowest concentration in which the color did not exhibit variation was taken as the MIC value. The standard compounds adopted as controls for the antimicrobial assays were amoxicillin (0.078-10 µg. mL^{-1}), streptomycin (0.078-10 µg. mL^{-1}) and nystatin (0.016-16 µg.mL^{-1}). The antimicrobial assay was performed in triplicate. Positive and negative controls were included.

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3 RESULTS AND DISCUSSION

3.1 ANTIOXIDANT ACTIVITY

The results of antioxidant activity are summarized in Table 1.

Table 1: Antioxidant activity determined by methods DPPH and Total phenolics in isoflavones extract raw and bioconverted.

Sample	DPPH (mg Trolox equivalent)	FRAP (mM Trolox equivalent)	Total phenolic contents (μg GAE/mL)
Raw extract	123.21	1378,7	273.3
Bioconverted Extract	1060.73	1969,9	418.5

According the results all antioxidant methods presented higher activity to bioconverted extract. The bioconverted extract (1060.73 mg Trolox equivalent) presented near 9 times superior antioxidant activity measured by DPPH method than raw extract (123.21 mg Trolox equivalent). The Total phenolic content presented similar behavior being higher to bioconverted extract than in raw extract.

3.2 ANTIMICROBIAL ACTIVITY

The antimicrobial activity of the isoflavones extracts raw and bioconverted against *S. aureus, C. albicans and E. coli* were higher than 1600 μg/mL in the range studied 0.78 to 1600 μg/mL.

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