Enzymatic Production of Gallic Acid Glycoside using Dextransucrase and Its Functional Characterization

Seung-Hee Nam[1]*, Jin-A Ko[2], Young-Min Kim [2, 3]

1 Institute of Agricultural Science and Technology, Chonnam National University, Gwangju 61186, South Korea.
2 Bio-energy Research Center, Chonnam National University, Gwangju 61186, South Korea
3 Department of Food Science & Technology and BK21 Plus Program, Chonnam National University, Gwangju 61186, South Korea.

Abstract

Gallic acid glucoside was synthesized via the acceptor reaction of a glucansucrase from Leuconostoc mesenteroides B-512FMCM with gallic acid and sucrose. The glucoside was purified by silica gel column chromatography and C18 reverse-phase high-performance liquid chromatography after removing unreacted gallic acid and sucrose using butanol and water (1:1, v/v). Gallic acid glucoside .was further confirmed by LC/MS/MS. Three variables, conc. of substrate, enzyme, and acceptor were used to determine the optimum producing condition of gallic acid glucoside with a five level central composite design and response surface methodology. Gallic acid glucoside exhibited slower effects on 1,1-diphenyl-2-picrylhydrazyl radical scavenging and NO scavenging and higher effects on anti-lipid per oxidation using chemiluminescent assay. However, its water solubility was increased dramatically by 180% than gallic acid.

Background & Objectives

Gallic acid, trihydroxybenzoic acid, is found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants (Reynolds and Wilson, 1991). GA is commonly used in the pharmaceutical industry due to its beneficial functions such as anti-fungal, anti-viral, antioxidant, and anticancer activities without harming healthy cells. GA is also used to treat albuminuria and diabetes (Fiuza, 2004).

Glucosyltransferases are enzymes that synthesize either dextrans or glucans, using sucrose as a substrate (Robyt and Martin 1983). Glucansucrases are also known to be able to transfer mono-,di-, or higher glucose units to other carbohydrate acceptors, via a variety of glycosidic linkages (Seo et al. 2005). In this study, we report the enzymatic synthesis of a set of GA-glc, using the glucansucraseisolated from L. mesenteroides 512FMCM. The enzymatically synthesized compound, GA-glc was enzymatically synthesized with 27% yield from total GA for the first time. GA-glc was purified using silica gel chromatography and C18 reverse-phase HPLC for identification its structure by LC/MS/MS. Production of GA-glc was optimizedby response surface method with 17 combinations of sucrose, enzyme, or GA concentrations. GA-glc exhibited lower antioxidant activity and nitric oxide scavenging activity and slightly higher effects on lipid peroxidation inhibition, compared to those of GA

Materials and Methods

Glycosyation of Gallic acid and its detection via TLC

The reaction mixture (250 mL) was consisted of 0.1M gallic acid, 80 mM sucrose and glucansucrase (2.4 units/mL). The mixture was incubated at 28 ˚C for 12 h, after which the sucrose had been depleted. The reaction mixture was then boiled for 5 min to halt the enzyme reaction. TLC was conducted at room temperature, using a silica gel TLC plate (Merck Co.). Plate was developed using a solvent mixture of ethyl acetate/acetic acid/water (3:1:1, v/v/v).

Purification, identification of GA-glc

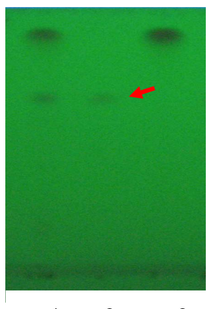
The reaction digests were then subjected to Silica gel column chromatography. The transfer products were washed indistilled water to remove the sugars and then successively eluted with 85% (v/v) acetonitrile. The eluant was then subjected to HPLC on an PDA-MD2015 instrument (JASCO). Response surface methodology was used to find the effects of sucrose, enzyme, GA on optimum GA –glc production.

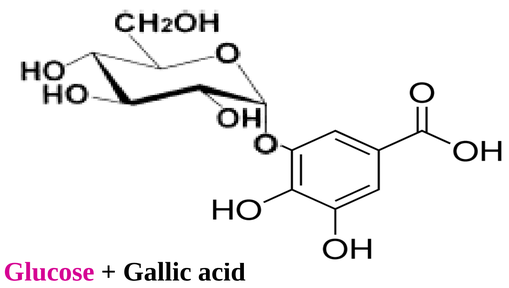
Functional characterization

GA and GA-glc was prepared to measure the their physiological function on antioxidant, anti-lipid peroxidation, NO scavenging effect. **Antioxidant activity** DPPH assay was used according to Blois (1958). Samples (0.05mL) and 1mM DPPH in ethanol (250uL) was measured at 517nm with a Microplate Reader (Molecular device). Vitamin C was used as internal standard..

Nitric oxide scavenging activity Sample (100uL) and 1mM sodium nitroprusside (200uL) were determined by the method of Sreejayan and Rao (1997), after adding of 2% acetic acid, Griess reagent (300uL). **Inhibition of lipid peroxidation** Anti-lipid peroxidation was measured using the chemiluminescence-measuring device HP-CLA (Tohoku). Antioxidant species in sample reacted with free radicals attached luminol in ample and delay the generation of photons until the antioxidant species are consumed. Sample (20uL) or vit E (20 uL of 1nM/uL) was applied to detect effect.

Results and Conclusions





Glucose + Gallic acid

Figure 1. Thin-layer chromatogram of the glucansucrase acceptor reaction digests: lane 1, enzyme reaction digest with GA; lane 2, glycoslyated GA; lane 3, GA only. Arrows indicate GA acceptor reaction products.

➢ GA and GA-glc were detected by UV245 after developing using a solvent mixture of ethyl acetate/acetic acid/water.

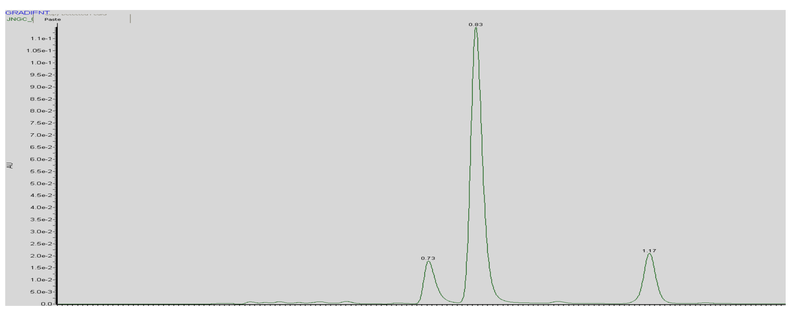


Figure 2. HPLC chromatogram of gallic acid glucoside after silica gel column chromatography. Column, *u*-Bondapak C18 (300 x 19 mm); the mobile phase was A (methanol containing 0.1% formic acid) and B (water containing 0.1% formic acid)

➢According to the HPLC chromatogram, GA-glc was purified using HPLC. The yields of GA-glc was 57% of total GA in reaction digest. Each fraction from HPLC was further confirmed by TLC.

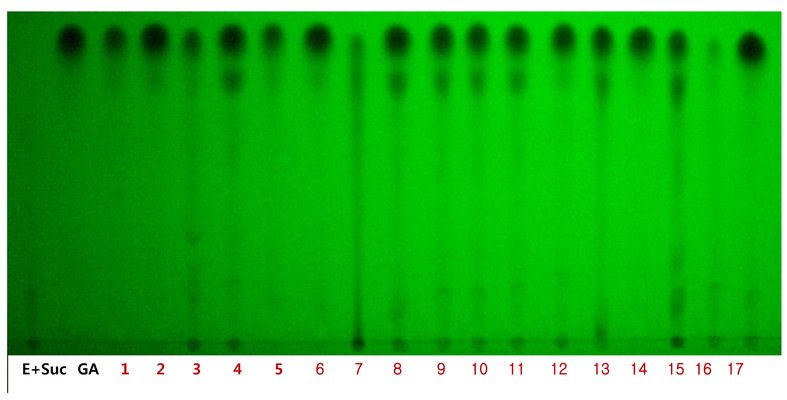


Figure 3. TLC analysis of gallic acid glycoside production of 17combination variables

➢ On 17 combinations of three factor (X1: substrate X2:Enzyme X3: Acceptor) and five levels (-2, - 1, 0, +1, +2), Enzyme (1-120U/mL), sucrose (10-720mM), and gallic acid (2.5-55mM) were applied to find optimum GA-glc producin condition. Density of GA-glc on TLC was used to final polynominal equation

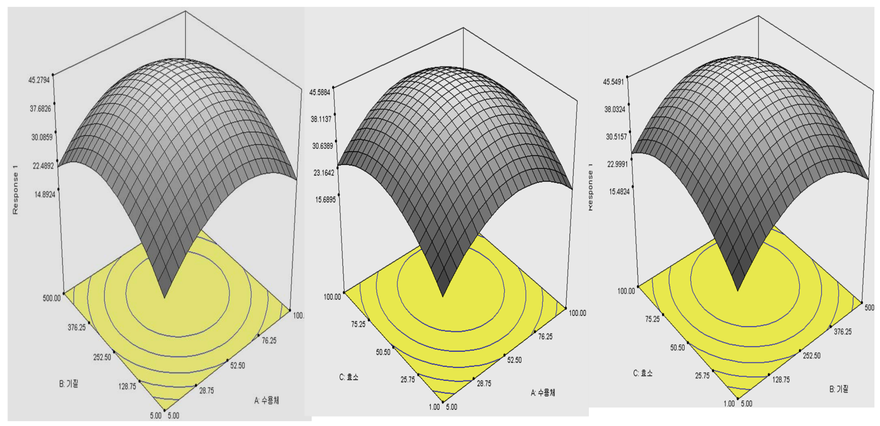


Figure 4. Three dimentional plot of the response surface for gallic acid glycoside production at 24 h

➢ Final Equation in Terms of Actual Factors Y= GA-glc product [mM]

$$Y = -0.71501 + 0.11597X_1 + 0.020079\ X_2 + 0.090799\ X_3 - 9.76303\ X_1^2 - 3.88640\ X_2^2 - 8.26857\ X_3^2 + 4.20695\ X_1X_2 - 1.43570X_1X_3 + 2.04061X_2X_3$$

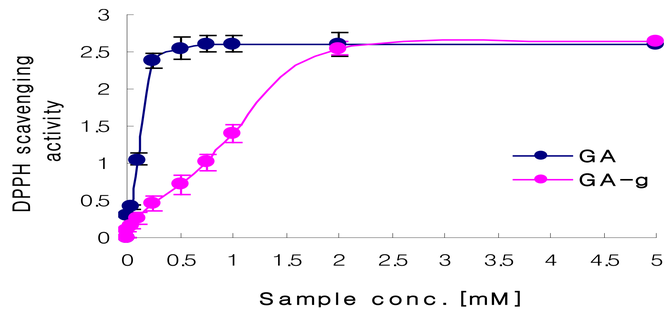
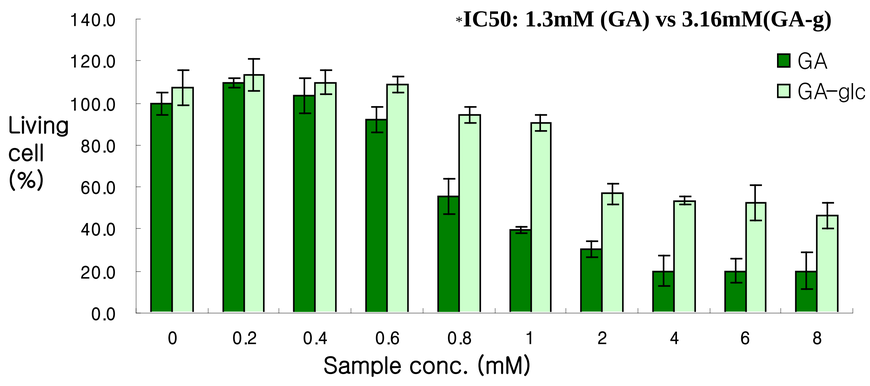


Figure 5. Functional characterizations of GA and GA-glc by DPPH free radical scavenging

➢ On RC50 of vitamin C is 0.41mM, RC50 of CA-g exhibited slighlty lower antioxidant activity with 1.07mM RC50, compared to that of CA with 0.73mM RC50.



➢ As a result of MTT assay with HT2 cell, GA-g exhibited lower anticancer activity with 3.16mM IC50, compared to that of GA with 1.3mM RC50 .

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This work was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (NRF-2016R1D1A1B03936148), Republic of Korea.