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Synthesis and characterization of hydroquinone fructoside using *Leuconostoc mesenteroides* levansucrase

Jin Kang · Young-Min Kim · Nahyun Kim · Du-Woon Kim · Seung-Hee Nam · Doman Kim

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Abstract Hydroquinone (HQ) functions as a skin-whitening agent, but it has the potential to cause dermatitis. We synthesized a HQ fructoside (HQ-Fru) as a potential skin-whitening agent by reacting levansucrase from *Leuconostoc mesenteroides* with HQ as an acceptor and sucrose as a fructofuranose donor. The product was purified using 1-butanol partition and silica-gel column chromatography. The structure of the purified HQ-Fru was determined by ^{1}H and ^{13}C nuclear magnetic resonance, and the molecular ion of the product was observed at *m/z* 295 $(C12\ H16\ O7\ Na)^{+}$. The HQ-Fru was identified as 4-hydroxyphenyl-β-D-fructofuranoside. The optimum condition for HQ-Fru synthesis was determined using a response surface method (RSM), and the final optimum condition was 350 mM HQ, 115 mM sucrose, and 0.70 U/ml levansucrase, and the final HQ-Fru produced was 1.09 g/l. HQ-Fru showed anti-oxidation activities and inhibition against tyrosinase. The median inhibition concentration (IC_{50}) of 1,1-diphenyl-2-picrylhydrazyl scavenging activity was 5.83 mM, showing higher antioxidant activity compared to β-arbutin (IC_{50}=6.04 mM). The K_i value of HQ-Fru (1.53 mM) against tyrosinase was smaller than that of β-arbutin (K_i=2.8 mM), indicating that it was 1.8-times better as an inhibitor. The inhibition of lipid peroxidation by HQ-Fru was 105.3% that of HQ (100%) and 118.9 times higher than that of β-arbutin (0.89% of HQ).

Keywords *Leuconostoc mesenteroides* · Levansucrase · Hydroquinone · Acceptor reaction · Fructosylation

J. Kang · Y.-M. Kim · D. Kim (✉)
School of Biological Sciences and Technology,
Chonnam National University,
Gwangju 500-757, Republic of Korea
e-mail: dmkim@jnu.ac.kr

N. Kim
Korean Minjok Leadership Academy,
Hoengseong 225-823, Republic of Korea

D.-W. Kim
Eco Marine Bio-Center,
Jeonnam 500-749, Republic of Korea

S.-H. Nam
Jeonnam Agricultural Research & Extension Services,
Jeonnam 520-715, Republic of Korea

Introduction

Melanin plays the main role in skin color and pigmentation (Riley 2003). Up to 10% of skin cells in the innermost layer of the epidermis produce melanin. Upon exposure of the skin to ultraviolet (UV) radiation, melanogenesis is initiated through the action of the enzyme tyrosinase (Parvez et al. 2006), one of the key enzymes in melanin synthesis. A number of natural and synthetic tyrosinase inhibitors have been reported, and attempts have been made to modify them to improve their functionality (Sugimoto et al. 2003). Hydroquinone (HQ), a part of β-arbutin, is a good general-purpose inhibitor, stabilizer, and antioxidant. However, the US Food and Drug Administration recently issued a proposal to ban HQ-containing over-the-counter and prescription products. HQ often results in inflammation of the skin, which has prompted a need for alternatives, including naturally occurring compounds (Pieroni et al. 2004). The traditional use of plants for cosmetic purposes is a common practice in the domestic medicine of many cultures, and may provide leads for better antipigmentation compounds.

For example, 4-hydroxyphenyl β-D-glucopyranoside (β-arbutin) found in the leaves of plants is used as a cosmetic ingredient. However, only a few natural compounds have been utilized as skin-whitening agents, principally due to a variety of safety concerns (Maeda and Fukuda 1991). Enzymatic transglycosylation using *Leuconostoc mesenteroides* glycansucrases has previously been applied to the modification of a variety of bioactive substances in an effort to improve their functionality. One study reported on the synthesis of epigallocatechin gallates and quercetin analogs using glucansucrases, with sucrose as a substrate (Moon et al. 2006). The glucosylated epigallocatechin gallate (10%–20% product yield) was quite stable to UV radiation and 50–100 times as soluble in water as compared to epigallocatechin gallate itself, which is very easily oxidized. However, the glucosylated epigallocatechin gallates exhibited similar or slower antioxidant effects, depending on their structures. 4-Hydroxyphenyl α-D-glucopyranoside (α-arbutin), or HQ fructoside (HQ-Fru), was also synthesized enzymatically by using sucrose phosphorylase or β-fructofuranosidase with HQ and sucrose, respectively, and α-arbutin showed stronger inhibitory effects on human tyrosinase than that of beta-arbutin (Sugimoto et al. 2003; Nakano et al. 2002).

A levansucrase catalyzes the transfer of the fructosyl residue from sucrose to various acceptor molecules. Three different reactions can be distinguished depending on the acceptor molecule: (1) hydrolysis of sucrose when water is used as acceptor, (2) transfer reaction when sucrose or gluco- and fructosaccharides are used as acceptor (oligosaccharide synthesis), and (3) polymerization when the growing fructan chain is used as acceptor (levan synthesis) (Hestrin et al. 1943; Hijum et al. 2001). Levansucrase produced by several microorganisms was capable of transferring fructofuranose from sucrose to many compounds (acceptors) containing hydroxyl group in the fields of cosmetics, foods, and pharmaceuticals (Belghith et al. 1996), as a hypocholesterolemic agent (Yamamoto et al. 2000), and an antitumor agent (De Roos & Katan 1988). *Leuconostoc mesenteroides* levansucrase gene (*m1ft,* GenBank accession no. AY665464) codes for a protein of 424 amino acid residues with a calculated molecular mass of 47.1 kDa (Kang et al. 2005). The *m1ft*-encoded enzyme M1FT produces erlose [*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside] as an acceptor product with maltose.

In this study, HQ-Fru, 4-hydroxyphenyl-β-D-fructofuranoside, was first synthesized via the acceptor reactions of levansucrase from *L. mesenteroides* with HQ and sucrose. HQ-Fru was purified and the structure was determined. Then, biochemical properties such as antioxidant activity, inhibition against tyrosinase, inhibition against lipid peroxidation, and nitrite scavenging activity were studied. The results indicate the promise of HQ-Fru as a functional cosmetic agent.

Materials and methods

Materials

HQ, β-arbutin, deuterium oxide (D_2O), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,5-dihydroxybenzoic acid, and tyrosinase (T7755) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Silica-gel (40–60 μm) was purchased from Acros Organics (Geel, Belgium). Other chemical reagents were commercially available and of chemical pure grade.

Preparation of enzyme

Escherichia coli BL21(DE3)pLysS containing the *mlft* gene on a pRSET expression vector from *L. mesenteroides* B-512 FMC was grown to midstationary phase at 37 °C with vigorous aeration in LB broth (Difco, Detroit, MI, USA) containing 50 mg/l ampicillin (Kang et al. 2005). The culture was induced by adding isopropylthio-β-D-galactoside at a concentration of 1 mM and incubating for 6 h at 28 °C. All subsequent steps were carried out at 4 °C. The cells were harvested, washed with 100 mM potassium phosphate (pH 7.4), centrifuged (5,000×*g*, 10 min), and then disrupted by sonication. The expressed protein was purified using Ni^{2+}–nitrilotriacetic acid (NTA)–agarose chromatography (Qiagen, Valencia, CA, USA). The lysate was incubated with a Ni^{2+}–NTA slurry at 4 °C for 1 h and the mixture was loaded to a column. The column was washed four times with washing buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0) and M1FT protein was eluted with 0.5 ml of elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0). Levansucrase was incubated with 20 mM Na-acetate buffer (pH 6.0) containing 100 mM sucrose at 30 °C. The glucose concentration liberated from sucrose was determined using a D-glucose oxidase enzymatic analysis kit (Boehringer Mannheim, Mannheim, Germany) or by thin layer chromatography (TLC). TLC analysis was done on silica gel coated glass plate (Merck, Darmstadt, Germany) with nitromethane–1-propanol–water (4:10:3, v/v/v) as a solvent system. After irrigation, the TLC plate was dried and visualized by dipping in a solution containing 0.3% (w/v) *N*-(1-naphthyl)-ethylenediamine and 5% (v/v) H_2SO_4 in methanol and by heating at 120 °C for 5 min (Mukerjea et al. 1996). One enzyme unit was defined as the amount of the enzyme releasing 1 μmol of glucose per minute, which is equal to the addition of 1 μmol fructose added to the acceptor per minute.

Fructosylation of HQ

The reaction mixture (1 l) in 50 mM Na-acetate (pH 5.2) consisting of 350 mM HQ, 115 mM sucrose, and M1FT protein (0.70 U/ml) was incubated at 28 °C for 6 h and placed in a boiling water bath for 10 min to halt the enzyme reaction. The fructosylation of HQ was confirmed using TLC analysis method as described above.

Purification of HQ-Fru

The reaction digest (1 l) was partitioned with the same volume of 1-butanol to obtain HQ-modified products from the 1-butanol layer. These were further concentrated under vacuum to 50 ml using a rotary evaporator (EYELA, Tokyo, Japan) at 65 °C. The concentrate was applied to a 3 × 62-cm silica gel column and purified HQ was eluted with 90% (v/v) acetonitrile. HQ-Fru purity was confirmed by high-pressure liquid chromatography using a LC-10 AD HPLC apparatus (Shimadzu, Kyoto, Japan) under the following conditions: TSK-GEL, amide-80 5 μm (Waters, Milford, MA, USA); mobile phase, acetonitrile/water = 75:25 (v/v); flow rate, 1.0 ml/min; room temperature; detection, RID-10A RI detector (Shimadzu).

Matrix-assisted laser desorption/ionization–time of flight mass spectrometry

Purified HQ-Fru (18 mg/ml) was diluted with deionized water then mixed 1:1 (v/v) with 2,5-dihydroxybenzoic acid (1 mg/ml) dissolved in water. The mixed solution (1 μl) was then spotted onto a stainless steel plate and slowly dried at room temperature. The mass spectrum was acquired using a Voyager DE-STR matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometer (Applied Biosystems, Foster City, CA, USA). The mass spectra were obtained in the positive linear mode with delayed extraction (average of 75 laser shots) with a 65-kV acceleration voltage.

Nuclear magnetic resonance spectroscopy

Approximately 18 mg of purified HQ-Fru was dissolved and evaporated three times with D_2O, and then placed into 3-mm nuclear magnetic resonance (NMR) tubes. NMR spectrum was obtained on a Unity Inova 500 spectrometer (Varian, Palo Alto, CA, USA) operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C at 25 °C. Spectra of homonuclear correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) were analyzed to ascertain the linkages.

Experimental design for optimization of acceptor reaction

The experimental RSM data were fitted via the response surface regression procedure using the following second-order polynomial equation (Box et al. 1978):

$$Y_i = \beta_0 + \sum_i \beta_i x_i + \sum_{ii} \beta_{ii} x_i^2 + \sum_{ij} x_i x_j$$

where Y_i is the predicted response, $x_i x_j$ are the independent variables, β_0 is the offset term, β_i is the i^{th} linear coefficient, β_{ii} is the i^{th} quadratic coefficient, and β_{ij} is the ij^{th} interaction coefficient. In this study, however, the independent variables were coded as X_1, X_2, and X_3. Thus, the second-order polynomial equation can be presented as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

Design-Expert 6.0.11 CCD RSM software (State-Ease, Minneapolis, MN, USA) was also used for regression analysis and graphical analysis of the data obtained during whole experiments. Analysis of variance (ANOVA) was used to estimate the statistical parameters. The second-order polynomial equation was employed to fit the experimental data. The significance of the model equation and model terms was evaluated by Fisher's test. The quality of fit for the polynomial model equation was expressed by the coefficient of determination (R^2) and adjusted R^2. The fitted polynomial equation was expressed as three-dimensional surface plots to show the relationship between the responses and the experimental levels of each variables used in the design. The combination of different optimized parameters producing maximum response was determined in an attempt to verify the validity of the model. From preliminary experiment, three factors (levansucrase unit, sucrose, and HQ concentrations) were selected to optimize the synthesis of HQ-Fru: levansucrase unit (U/mL), 0.2–1.2; sucrose concentration (mM), 0–274.7 mM; and HQ concentration (mM), 0–770.5.

Antioxidant effects

The antioxidant activities of HQ, HQ-Fru, and β-arbutin were assessed using DPPH. Each sample (0.1, 0.5, 1, 5, 10, 25, 50, and 100 μM) was dissolved in 100 μl ethanol and mixed thoroughly with 100 μM DPPH in ethanol solution (total volume 200 μl). After 10 min at room temperature in darkness, the absorbance of each mixture was measured at 517 nm on a microplate reader (Molecular Devices, Sunnyvale, CA, USA). DPPH radical-scavenging activity was determined according to the decrease in absorbance of

the DPPH radical in the sample and compared to that observed with an ethanol blank (Borek 2001).

Tyrosinase inhibition

The reaction mixture (90 μl) contained 3.3 mM 3-(3, 4-dihydroxyphenyl)-L-alanine (DOPA) in 330 mM phosphate buffer (pH 7) and enzyme in the presence or absence of inhibitors. Fifteen units of tyrosinase was used to determine the K_i value. To determine which type of inhibition occurred, a Dixon plot of the relationship between the reciprocal of the velocity and the HQ-Fru concentration (0, 0.1, 0.5, and 1 mM) was made using four substrate concentrations of 0.1, 0.5, 1, and 5 mM DOPA. The reaction mixtures in 96-well plates were incubated at 37 °C for 10 min, and the absorbance was measured at 475 nm in a microplate reader (Funayama et al. 1995).

Anti-lipid peroxidation activity

Anti-lipid peroxidation activity was measured using a HP-CLA chemiluminescence-measuring device (Tohoku Electronic Industrial, Tokyo, Japan) and an ARA-L kit (ABCD GmbH, Berlin, Germany). Antioxidant species in each sample (HQ, HQ-Fru, or β-arbutin) was incubated with ample free radical-attached luminol to delay the generation of photons until the antioxidant species were consumed. The lag time(s) was (were) proportional to the amount of antioxidant species in each sample. Each sample (20 μl) or vitamin E (20 μl of 1 μM/ml) was applied to detect its antioxidant effect (Sreejayan et al. 1997).

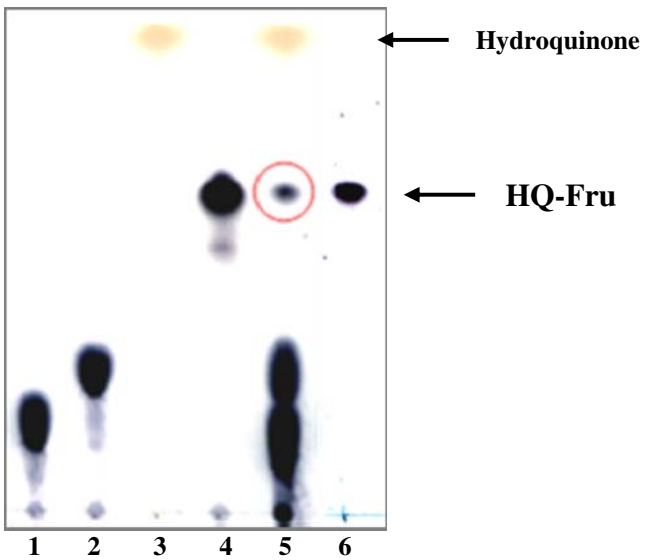

Fig. 1 Thin-layer chromatogram of the levansucrase acceptor reaction products. *Lane 1*, sucrose; *lane 2*, glucose; *lane 3*, standard HQ; *lane 4*, standard β-arbutin; *lane 5*, levansucrase reaction digest; *lane 6*, purified HQ-Fru

Table 1 ^{13}C and ^{1}H NMR data of HQ-Fru (ppm)

Carbon atom position	HQ-Fru	
	δ_C	δ_H (*J*, Hz)
C-HQ		
1	152.07	
2,6	123.68	7.11–7.13 (dd) (*J* = 2.5, 2)
3,5	115.74	6.84–6.86 (dd) (*J* = 2.5, 2.5)
4	146.07	
C-Fru		
1′	60.16	3.60 (d) (*J* = 12.5)
		3.69–3.71 (m)
2′	105.96	–
3′	75.98	4.31 (d) (*J* = 8.5)
4′	74.55	4.18–4.19 (t) (*J* = 8, 8)
5′	81.66	3.98–3.99 (dt) (*J* = 3, 3, 3)
6′	62.62	3.81–3.83 (dd) (*J* = 3, 3.5)
		3.69–3.71 (m)

Nitrite scavenging activity

The nitrite scavenging activity of HQ-Fru was determined as previously described (Muller 2000). HQ-Fru sample (100 μl of 5 mM) and 1 mM Na-nitroprusside (200 μl) were mixed and incubated at room temperature for 30 min. After addition of 2% acetic acid, Griess reagent (300 μl; 0.2% naphthylenediamine dihydrochloride and 2% sulphanilamide in 5% phosphoric acid) was added to measure the remaining nitrite content. Nitrite radical scavenging activity was determined according to the decrease in absorbance of

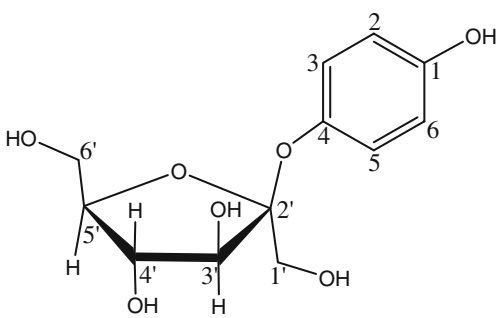

Fig. 2 The structure of HQ-Fru

Table 2 Experimental codes, ranges, and levels of the independent variables for RSM experiment

Variables	Units	Symbol code	Levels				
			−1.682[a]	−1	0	+1	+1.682[a]
Enzyme	(U/ml)	X_1	0.2	0.4	0.7	1.0	1.2
Sucrose	mM	X_2	0.0	20.0	115.0	210.0	274.7
HQ	mM	X_3	0.0	100.0	350.0	600.0	770.5

$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$

[a] Based on program design value

the nitrite in the sample as compared to that observed with a blank (Sreejayan and Rao 1997).

Results

Synthesis and purification of HQ-Fru

After the acceptor reaction involving *L. mesenteroides* M1FT levansucrase with HQ and sucrose, we were able to identify a reaction product via TLC showing the same running characteristics as the HQ-Fru control, indicating this product as a HQ-Fru (Fig. 1, lane 5). The maximum conversion via TLC showing the yields of HQ-Fru was 14.0% with 20 mM sucrose reacted or 2.5% with 100 mM HQ reacted. However, the final optimum condition for HQ-Fru synthesis was 350 mM HQ, 115 mM sucrose, and 0.70 U/ml levansucrase and resulted in 1.09 g/l of HQ-Fru. The acceptor reaction mixture was purified using both 1-butanol partitioning and silica gel column chromatography. The purified HQ-Fru showed a single band on a HPLC chromatogram (data not shown). The yield of HQ-Fru purification was 134 mg (12.3% of total HQ-Fru synthesized). The purified HQ-Fru was obtained as a yellowish-white powder.

Structural determination of HQ-Fru

The number of fructose units attached to the purified HQ-Fru was confirmed using MALDI-TOF MS, which showed a single fructose attachment (data not shown). The fructosidic linkage for HQ-Fru was determined using ^{1}H, ^{13}C, ^{1}H-COSY, HSQC, and HMBC analyses; the results are shown in Table 1. The molecular ion of HQ-Fru was observed at *m/z* 295 $(C12\ H16\ O7\ Na)^{+}$. In Table 1, a doublet signal at 3.60 ppm (J = 12.5 Hz) was assigned to the anomeric proton, thereby indicating that only one fructosyl residue was β-linked to HQ. Ten signals were observed by ^{13}C-NMR analysis (Table 1). Four of them (152.07–115.74 ppm) were assigned to carbons of HQ, and six (105.96–60.16 ppm) to carbons of fructose (Nakano et al. 2002). From these results, it was confirmed that HQ-Fru was 4-hydroxyphenyl-β-D-fructofuranoside (HQ-Fru) (Fig. 2). Thus, the linkage between HQ and fructofuranoside by the levansucrase acceptor reaction was beta.

Determination of polynomial equation coefficients for optimum HQ-Fru synthesis

The design matrix and the corresponding results of RSM experiments are shown in Table 2, along with the mean predicted values. The selected culture conditions including levansucrase unit (X_1), sucrose concentration (X_2), and HQ concentration (X_3) were investigated. RSM was used to study the interaction of these variables within a range of −1.682 to +1.682 in relation to HQ-Fru synthesis (Table 2).

Table 3 CCD matrix for the experimental design and predicted responses for HQ-Fru synthesis

Run No.	Coded levels			HQ-Fru synthesis (mM)	
	X_1	X_2	X_3	Actual	Predicted
1	0.7	−44.77	350.0	0.00	0.39
2	0.7	115.0	350.0	4.00	3.99
3	0.7	115.0	350.0	4.00	3.99
4	0.7	115.0	350.0	4.00	3.99
5	0.7	274.7	350.0	3.50	3.37
6	0.4	20.0	600.0	1.00	0.71
7	0.7	115.0	−70.45	0.00	−0.15
8	0.7	115.0	350.0	4.00	3.99
9	0.4	20.0	100.0	0.30	0.14
10	0.2	115.0	350.0	3.00	3.18
11	1.0	20.0	600.0	2.80	2.46
12	0.7	115.0	350.0	4.00	3.99
13	1.0	20.0	100.0	1.00	0.99
14	1.0	210.0	600.0	3.50	3.48
15	0.4	210.0	100.0	2.50	2.66
16	0.7	115.0	770.5	1.50	1.90
17	0.7	115.0	350.0	4.00	3.99
18	1.0	210.0	100.0	1.50	1.61
19	0.4	210.0	600.0	3.80	3.63
20	1.2	115.0	350.0	3.70	3.77

$Y = -3.024 + 4.285 X_1 + 0.038 X_2 + 0.012 X_3 - 2.027 X_1^2 - 0.000082 X_2^2 - 0.0000176 X_3^2 - 0.016 X_1 X_2 + 0.003 X_1 X_3 - 0.0000042 X_2 X_3$

Table 4 ANOVA for the RSM parameters fitted to second-order polynomial equation

Source	Sum of squares	Degree of freedom	Mean square	*F* value	*P* value > *F*
Model	41.91	9	4.66	68.11	<0.0001
Residual	0.68	10	0.068		
Lack of fit	0.68	5	0.14		
Pure error	0	5	0		
Cor Total	42.59	19			

Standard deviation=0.26, R^2 = 0.9839, CV = 10.04, Adj-R^2 = 0.9695

A total of 20 experiments were performed with different combinations of these factors (Table 3). HQ-Fru synthesis varied with changes of levansucrase units, sucrose concentration, and HQ concentration. As ascertained from the central points of the corresponding contour plots, the three variables were 0.70 U/ml levansucrase activity, 115 mM sucrose, and 350 mM HQ. From ANOVA analysis, values of "Prob > F" <0.05 indicate model terms are significant. In this case, $X_2, X_3, X_1^2, X_2^2, X_3^2, X_1X_2$, and X_1X_3 were significant model terms (Table 4). The model *F* value was 68.11, implying that the model was significant. The regression equation coefficients were calculated and the data was fitted to a second-order polynomial equation. The response, HQ-Fru synthesis by levansucrase acceptor reaction, was expressed in terms of the following regression equation:

$$Y = -3.024 + 4.285X_1 + 0.038X_2 + 0.012X_3 - 2.027X_1^2 - 0.0000828X_2^2 - 0.0000176X_3^2 - 0.016X_1X_2 + 0.003X_1X_3 - 0.0000042X_2X_3$$

where X_1 is levansucrase unit (U/ml), X_2 is sucrose concentration (mM), and X_3 is HQ concentration (mM). The regression equation obtained from ANOVA indicated a R^2 (multiple correlation coefficient) value of 0.9839 (a value >0.75 indicates fitness of the model). This was an estimate of the fraction of overall variation in the data accounted by the model and, thus, the model was capable of explaining 98.39% of the variation in response. The "adequate precision value" of the present model was 22.40, suggesting that the model can be used to navigate the design space. The "adequate precision value" is an index of the signal-to-noise ratio, and values exceeding four are desirable prerequisites for a model to be a good fit. Based on the model, the predicted response for HQ-Fru synthesis was 3.99 mM, and the observed experimental value was 4 mM at 0.70 U/ml levansucrase, 115 mM sucrose, and 350 mM HQ, representing near identical results from the predicted and actual HQ-Fru synthesis.

Antioxidant activity

HQ and HQ-Fru show different antioxidant activities depending on their structural configurations (Table 5). The median inhibition concentration (IC_{50}) value of HQ-Fru based on DPPH radical scavenging activity was 5.83 mM, indicative of lower activity as compared with that of HQ (IC_{50}=0.33 mM). However, it showed a little higher antioxidant activity compared to β-arbutin (6.04 mM), which is a commercial antioxidant cosmetic compound. This result indicates that the attachment of fructose on HQ decreased its antioxidant activity in vitro, while maintaining good potential as an antioxidant ingredient of cosmetics.

Inhibition effect of lipid peroxidation

Reactive oxygen species (ROS) are continuously produced at a high rate as a byproduct of aerobic metabolism. ROS has been linked to aging and various diseases (Muller

Table 5 Effects of HQ, β-arbutin, and HQ-Fru on DPPH radical scavenging activity, nitrite scavenging activity, and inhibition of lipid peroxidation

Compounds	DPPH radical scavenging activity IC_{50} (mM)	Nitrite scavenging activity	Tyrosinase inhibitor K_i (mM)	Lipid peroxidation activity U[a]
HQ	0.33 ± 0.02	3.22 ± 0.2	–	37.26 ± 1.3
β-Arbutin	6.04 ± 0.2	27.09 ± 1.2	2.8 ± 0.1	0.33 ± 0.04
HQ-Frc	5.83 ± 0.2	14.81 ± 1.6	1.53 ± 0.05	39.24 ± 1.9

[a] CL.arb.U = chemiluminant absorbance unit

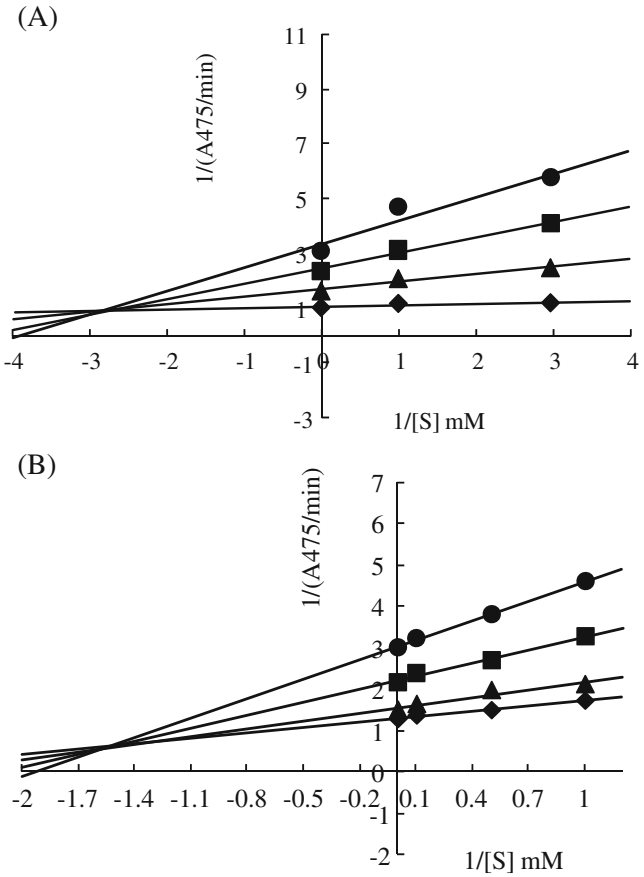

Fig. 3 Dixon plots showing the reciprocal of the velocity (1/υ) of the mushroom tyrosinase reaction vs inhibitor of β-arbutin (**A**) or HQ-Fru (**B**) in the presence of 0–1 mM concentrations. Various substrate concentrations of L-DOPA (0.1, 0.5, 1.0, 5.0 mM) were applied to obtain the Dixon plots. A_{475} represents an increase in the absorbance at 475 nm. Concentrations of L-DOPA: 0.1 mM (*circles*), 0.5 mM (*squares*), 1.0 mM (*triangles*), and 5.0 mM (*diamonds*)

2000). HQ exhibited varying antioxidant effects, depending on the structural configuration. HQ, HQ-Fru and β-arbutin exhibited different inhibition of lipid peroxidation (Table 5). The chemiluminescent absorbance unit of HQ-Fru was 39.24 U. Interestingly, HQ-Fru showed even higher inhibition activity compared to those of HQ (37.36 U) and β-arbutin (0.33 U).

Effect of nitrite scavenging activity

Nitrite scavenging activity of HQ-Fru was investigated (Table 5). The IC_{50} value of nitrite scavenging activity was 14.81 mM. This value of HQ-Fru indicated a lower scavenging activity than that of HQ (IC_{50}=3.22 mM) but a superior scavenging activity than that of β-arbutin (IC_{50}= 27.09 mM).

Tyrosinase inhibition

To observe the tyrosinase inhibition activity compared with β-arbutin, Fig. 3 shows the tyrosine-inhibitory effects exerted by HQ-Fru. The type of inhibition for HQ-Fru was identified as mixed noncompetitive type. The K_i value of HQ-Fru was 1.53 mM, showing higher tyrosinase inhibitor activity as compared with β-arbutin (2.8 mM).

Discussion

In this study, a HQ-Fru was synthesized via the acceptor reaction of a levansucrase from *L. mesenteroides* with HQ and sucrose and the production condition of HQ-Fru was optimized. There have been many efforts to synthesize the analog or derivatives of β-arbutin by glucosylation such as glucansucrase (Moon et al. 2006) and glycosidase (Sugimoto et al. 2003; Jun et al. 2008). To our knowledge, fructosylation of HQ with levansucrase is the first case. The modified HQ was purified and identified as 4-hydroxyphenyl-β-D-fructofuranoside by ^{13}C-NMR and ^{1}H-NMR analyses and TOF-MS as Nakano et al (2002). From the optimized condition, 1.09 g/l of HQ-Fru could be produced from HQ, sucrose, and levansucrase. Our results suggest that HQ is a suitable acceptor for the transfructosylation of levansucrase. Resultant HQ-Fru is another functional derivative of β-arbutin displaying strong inhibition activity of lipid peroxidation and antioxidant activity.

A great deal of effort has been expended in the synthesis of tyrosinase inhibitors such as arbutin derivatives (Sugimoto et al. 2003, 2005). In previous studies, arbutin displayed a dose-dependent inhibitory effect on the oxidation of L-DOPA catalyzed by mushroom tyrosinase with an IC_{50} of 8.4 mM (Funayama et al. 1995), 24 mM (Nihei et al. 2004), and 5.4 mM (Jin et al. 1999); this inhibition was described as competitive (Tomita et al. 1990; Jin et al. 1999) or noncompetitive (Funayama et al. 1995). Besides the transglucosylated products of arbutin, 4-hydroxy-phenyl β-maltoside and 4-hydroxy-phenyl β-maltotrioside exhibit stronger inhibitory activities than β-arbutin against human tyrosinase. The inhibitory activities of 4-hydroxy-phenyl α-maltoside and 4-hydroxy-phenyl α-maltotrioside were weakened by transglucosylation similar to the fructosylation of HQ. HQ-Fru showed increased DPPH scavenging activity, inhibition of tyrosinase, inhibition of lipid peroxidation, and nitrite-scavenging activity compared to β-arbutin, which is a commercial ingredient in various cosmetics. Thus, HQ-Fru would be a more suitable functional component in cosmetics. Inhibitory effects of HF on melanin synthesis in human melanoma cells are currently in progress.

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