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Facile synthesis of alkyl β-D-glucopyranosides from D-glucose and the corresponding alcohols using fruit seed meals

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Abstract

Various alkyl β-D-glucopyranosides were synthesized via a very simple procedure, by using *Prunus dulcis* (almond) kernel meal as an inexpensive biocatalyst. The *P. dulcis* (almond) meal is more robust than commercially available one and recyclable. Some popular fruits seed, including *Prunus persica* (peach), *Prunus armeniaca* (apricot), *Malus pumila* (apple), and *Eriobotrya japonica* (loquat), were tested as potential sources of the glucosidase in the form of meal. It was found that *P. persica* kernel meal and *M. pumila* seed meal not only had higher activity but also showed some complementary substrate specificities to that of almond β-glucosidase.

Keywords: Almond meal; Fruit seed meal; β-Glucosidase; Reverse hydrolysis

1. Introduction

Alkyl β-D-glucopyranosides are useful building blocks in carbohydrate synthesis [1–4]. Moreover, alkyl β-glucosides are a group of nonionic surfactants that may be used in cosmetics, pharmaceuticals, and kitchen detergents [5,6]. Considerable effort has been directed toward the development of strategies for glycosidation, with enzymatic methods attracting especial interest, by virtue of their mildness, high selectivity, and acceptance of unprotected sugars as substrates [7–9]. The ability of β-glucosidase for synthesis of alkyl β-glucosides from glucose and corresponding alcohols in one step has made this enzyme attractive for synthetic application [10–20] (Scheme 1). However, the high cost, instability, and the relatively narrow aglycone specificity of the commercial preparation of almond β-glucosidase become drawbacks on large-scale production. Herein, we wish to report a facile, cheap, and practical biotransformation for the synthesis of anomerically pure glucosides, which is functionally similar to that of the commercial preparation of almond glucosidase, but does not suffer from the drawbacks mentioned above.

In our previous work, we developed an efficient catalytic process using a defatted almond meal in a micro-aqueous organic solvent system for the formation of chiral cyanohydrins in high enantioselectivities and high yields [21–23]. Additionally, this reaction system has been applied to a column-type reactor to continuously produce the desired cyanohydrins in high throughput [24].

Almond meal not only is a rich source of oxynirilase but also exhibits high glucosidase activity [25]. Therefore, we envisioned that almond meal could also be used as a biocatalyst for glucosidation.

2. Experimental

2.1. Analytical methods

NMR spectra were recorded on a Bruker AM 300 spectrometer (300 MHz). Mass spectra were recorded on a Bruker APEXIII 7.0 TESLA FTMS using ESI mode. Optical rotations were measured using Perkin-Elemer 241 MC polarimeter. Column chromatography was carried out using silica gel (300–400 mesh).

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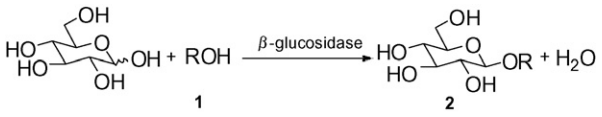


Scheme 1. Synthesis of alkyl β-glucosides based on reverse hydrolysis.

2.2. Materials and methods

Prunus communis L. var. *dulcis* Borkh (Sweet almond) was obtained from Xinjiang province, China. *Prunus persica* L. var. *scleropersica* (Reich) Yü et Lu (Peach), *Prunus armeniaca* Lam. var. *ansu* (Maxim.) Yü et Lu (red apricot), *Malus pumila* Mill (apple), *Eriobotrya japonica* (Thund.) Lindl. (loquat), and *Pyrus ussuriensis* Maxim. (pear) were purchased from the supermarket in Shanghai as fresh fruits. All other chemicals were of the highest purity commercially available and used without further purification if not mentioned. Almond β-glucosidase (EC 3.2.1.21) was purchased from Sigma Chemical Co. (G-0395, activity, 4 U/mg, one unit will liberate 1.0 μmol of glucose from salicin per min at pH 5.0 and 37 °C). The hydrolysis activity was 5.9 U/mg when it was determined using *p*-NPG as substrate.

The β-glucosidase activity was determined by measuring the release of *p*-nitrophenol from *p*-NPG (*p*-nitrophenyl β-D-glucopyranoside), one unit of β-glucosidase activity (U) is defined as the amount of enzyme that release 1 μmol *p*-nitrophenol per min. All samples were assayed in potassium phosphate buffer (50 mM, pH 7.0) at 50 °C under conditions that activity was proportional to enzyme concentration. A control test without enzyme was included. Protein was determined according to Bradford method using ovoalbumin as a standard [26].

2.3. The preparation of fruit seed meal

The *P. dulcis* (almond) kernels were soaked in distilled water for 2 h, peeled, air-dried, and then powdered in cold (0 °C) ethyl acetate with a homogenizer. The powder was defatted by further three washes with ethyl acetate, and two washes with acetone, then stored at 4 °C. The fresh fruit kernels or seeds were peeled and treated in the same manner.

2.4. General procedure for fruit seed meal catalyzed glucosylation

Glucose was dissolved in corresponding alcohols containing 10% (v/v) of water, and the fruit seed meal was then added. The mixture was stirred for 48–72 h at 50 °C, then filtered and concentrated under vacuum. The resultant syrup was applied to flash column chromatography (eluent EtOAc/MeOH = 15–10/1 or CH_2Cl_2/MeOH = 5/1). The corresponding β-D-glucopyranosides were collected as white solid or clear syrup.

Treat of glucose (0.45 g, 2.5 mmol) with *Malus pumila* seed meal (0.30 g) in ethanol/water (5 mL, 9:1, v/v) according to the general procedure gave ethyl β-D-glucopyranoside (**2a**) as clear syrup (0.423 g, 72%). $[\alpha]_D^{20}$ −38.5 (c 1.20, CH_3OH) ($[\alpha]_D^{20}$ −35.7, literature [27]); ^{1}H NMR (300 MHz, D_2O), δ/ppm: 1.8 (t, *J* = 6.9 Hz, 3H), 3.04 (t, *J* = 9 Hz, 1H), 3.13–3.31 (m, 3H), 3.48–3.55 (m, 2H), 3.70–3.80 (m, 2H), 4.26 (d, *J* = 8.1 Hz, 1H); ESIMS *m/z*: 231.1 [M + Na^+].

Treat of glucose (0.45 g, 2.5 mmol) with *M. pumila* seed meal (0.30 g) in propranol/water (5 mL, 9:1, v/v) according to the general procedure gave propranyl β-D-glucopyranoside (**2b**) as clear syrup (0.358 g, 66%). $[\alpha]_D^{20}$ −35.2 (c 1.40, CH_3OH) ($[\alpha]_D^{20}$ −39.5, literature [28]); ^{1}H NMR (300 MHz, D_2O), δ/ppm: 0.78 (t, *J* = 7.8 Hz, 3H), 1.46–1.53 (m, 2H), 3.12 (t, *J* = 8.1 Hz, 1H), 3.24–3.38 (m, 3H), 3.46–3.61 (m, 3H), 3.71–3.81 (m, 2H), 4.33 (d, *J* = 7.8 Hz, 1H); ESIMS *m/z*: 240.1 [M + NH_4^+].

Treat of glucose (0.50 g, 2.8 mmol) with *P. persica* kernel meal (0.60 g) in butanol/water (10 mL, 9:1, v/v) according to the general procedure gave butyl β-D-glucopyranoside (**2c**) as white solid (0.284 g, 46%). $[\alpha]_D^{20}$ −36.5 (c 0.76, CH_3OH) ($[\alpha]_D^{20}$ −37.4, literature [28]); ^{1}H NMR (300 MHz, D_2O), δ/ppm: 0.79 (t, *J* = 7.5 Hz, 3H), 1.22–1.32 (m, 2H), 144–1.54 (m, 2H), 3.14 (t, *J* = 8.7 Hz, 1H), 3.22–3.40 (m, 3H), 3.52–3.63 (m, 2H), 4.34 (d, *J* = 8.1 Hz, 1H); ESIMS *m/z*: 254.2 [M + NH_4^+].

Treat of glucose (0.54 g, 3 mmol) with *P. dulcis* kernel meal (0.60 g) in hexanol/acetonitrile/water (10 mL, 45:45:10, v/v/v) according to the general procedure gave hexyl β-D-glucopyranoside (**2d**) as white solid (0.130 g, 16%). $[\alpha]_D^{20}$ −33.9 (c 0.91, CH_3OH) ($[\alpha]_D^{20}$ −34.5, literature [28]); ^{1}H NMR (300 MHz, D_2O), δ/ppm: 0.76 (t, *J* = 6.9 Hz, 3H), 1.18–1.27 (m, 6H), 1.46–1.53 (m, 2H), 3.14 (t, *J* = 7.2 Hz, 1H), 3.23–3.40 (m, 3H), 3.52–3.63 (m, 2H), 3.77–3.85 (m, 2H), 4.33 (d, *J* = 7.8 Hz, 1H); ESIMS *m/z*: 287.1 [M + Na^+].

Treat of glucose (0.54 g, 3 mmol) and octanol (4.7 g, 36 mmol) with *P. dulcis* kernel meal (0.60 g) in acetonitrile/water (10 mL, 9:1, v/v) according to the general procedure gave octanyl β-D-glucopyranoside (**2e**) as white solid (0.105 g, 12%). $[\alpha]_D^{20}$ −31.7 (c 1.16, CH_3OH) ($[\alpha]_D^{20}$ −34.0, literature [28]); ^{1}H NMR (300 MHz, D_2O), δ/ppm: 0.84 (t, *J* = 4.2 Hz, 3H), 1.25 (m, 10H), 1.61 (m, 2H), 3.23 (t, 3.6 Hz, 1H), 3.36–3.45 (m, 3H), 3.57 (dd, *J* = 6.9, 16.5 Hz, 1H), 3.74 (dd, *J* = 4.2, 12 Hz, 1H), 3.86 (dd, *J* = 10.2, 17.7 Hz, 1H), 4.34 (d, *J* = 8.1 Hz, 1H); ESIMS *m/z*: 310.1 [M + NH_4^+].

Treat of glucose (0.27 g, 1.5 mmol) and dodecanol (2.0 g, 11 mmol) with *Malus pumila* seed meal (0.30 g) in acetonitrile/water (5 mL, 9:1, v/v) according to the general procedure gave dodecanyl β-D-glucopyranoside (**2f**) as white solid (0.033 g, 6%). $[\alpha]_D^{20}$ −25.5 (c 1.40, CH_3OH) ($[\alpha]_D^{20}$ −23.9, literature [29]); ^{1}H NMR (300 MHz, CD_3OD), δ/ppm: 0.81 (t, *J* = 6.9 Hz, 3H), 1.20–1.32 (m, 18H), 1.51–1.55 (m, 2H), 3.08 (t, *J* = 8.1 Hz, 1H), 3.16–3.26 (m, 3H), 3.43–3.48 (m, 1H), 3.58 (dd, *J* = 2.1, 6.9 Hz, 1H), 3.76–3.83 (m, 2H), 4.16 (d, *J* = 7.8 Hz, 1H); ESIMS *m/z*: 366.3 [M + NH_4^+].

Treat of glucose (0.54 g, 3 mmol) with *P. dulcis* kernel meal (0.60 g) in propan-2-ol/water (10 mL, 9:1, v/v) according to the general procedure gave isopropyl β-D-glucopyranoside (**2g**) as white solid (0.147 g, 29%). $[\alpha]_D^{20}$ −44.5 (c 1.16, CH_3OH) ($[\alpha]_D^{20}$ −34.4, literature [27]); ^{1}H NMR (300 MHz, D_2O), δ/ppm: 1.07 (dd, *J* = 6, 10.8 Hz, 6H), 3.06 (t, *J* = 9 Hz, 1H), 3.24 (t, *J* = 9.6 Hz, 1H), 3.28–3.36 (m, 2H), 3.55 (dd, *J* = 6, 12.3 Hz, 1H), 3.75 (dd, *J* = 1.8, 12.0 Hz, 1H), 3.96 (m, 1H), 4.38

(d, J = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO), δ/ppm: 21.8, 23.6, 61.2, 69.9, 70.2, 73.5, 76.8, 76.9, 101.0; ESIMS m/z: 245.1 [M + Na^+].

Treat of glucose (0.54 g, 3 mmol) with *Malus pumila* seed meal (0.60 g) in cyclohexanol/water (10 mL, 9:1, v/v) according to the general procedure gave cyclohexyl β-D-glucopyranoside (**2h**) as white solid (0.091 g, 11%); $[\alpha]_D^{20}$ −40.1 (c 0.90, CH_3OH) ($[\alpha]_D^{20}$ −46.3, literature [30]); ^{1}H NMR (300 MHz, D_2O), δ/ppm: 1.09–1.29 (m, 5H), 1.43–1.47 (m, 1H), 1.63–1.65 (m, 2H), 1.84–1.87 (m, 2H), 3.12 (t, J = 8.4 Hz, 1H), 3.24–3.41 (m, 4H), 3.58–3.78 (m, 2H), 3.81 (d, J = 10.5 Hz, 1H), 4.48 (d, J = 8.1 Hz, 1H); ESIMS m/z: 280.1 [M + NH_4^+].

Treat of glucose (0.54 g, 3 mmol) with *P. dulcis* kernel meal (0.60 g) in allylic alcohol-water (10 mL, 9:1, v/v) according to the general procedure gave allyl β-D-glucopyranosides (**2i**) as white solid (0.363 g, 46%). $[\alpha]_D^{20}$ −45.2 (c 0.885, CH_3OH) ($[\alpha]_D^{20}$ −39.1, literature [18]); ^{1}H NMR (300 MHz, D_2O), δ/ppm: 3.14 (t, J = 8.7 Hz, 1H), 3.20–3.37 (m, 3H), 3.58 (dd, J = 5.7, 12 Hz, 1H), 3.77 (dd, J = 2.1, 12.3 Hz, 1H), 4.08 (dd, J = 6.6, 12.3 Hz, 1H), 4.25 (dd, J = 5.7, 13.2 Hz, 1H), 4.36 (d, J = 8.1 Hz, 1H), 5.12–5.27 (m, 2H), 5.76–5.88 (m, 1H); ^{13}C NMR (75 MHz, DMSO), δ/ppm: 61.1, 68.8, 70.0, 73.47, 76.73, 76.89, 102.09, 116.50, 134.96; ESIMS m/z: 238.1 [M + NH_4^+].

Treat of glucose (0.27 g, 1.5 mmol) with *P. dulcis* kernel meal (0.30 g) in pent-4-en-1-ol-water (5 mL, 9:1, v/v) according to the general procedure gave 4-pentenyl β-D-glucopyranoside (**2j**) as white solid (0.170 g, 45%). $[\alpha]_D^{20}$ −35.7 (c 1.75, CH_3OH) ($[\alpha]_D^{20}$ −31.2, literature [31]); ^{1}H NMR (300 MHz, D_2O), δ/ppm: 1.56–1.65 (m, 2H), 1.99–2.06 (m, 2H), 3.13 (dd, J = 8.4, 9.0 Hz, 1H), 3.22–3.39 (m, 3H), 3.53–3.62 (m, 2H), 3.77–3.85 (m, 2H), 4.33 (d, J = 7.8 Hz, 1H), 4.88–5.00 (m, 2H), 5.75–5.84 (m, 1H); ESIMS m/z: 271.1 [M + Na^+].

Treat of glucose (0.27 g, 1.5 mmol) with *P. dulcis* kernel meal (0.30 g) in but-3-en-2-ol/water (10 mL, 9:1, v/v) according to the general procedure gave 2-butenyl β-D-glucopyranoside (**2k**) as clear syrup (0.087 g, 25%). $[\alpha]_D^{20}$ −43.7 (c 2.30, CH_3OH) ($[\alpha]_D^{20}$ −38.2, literature [28]); ^{1}H NMR (300 MHz, D_2O): δ 1.19 (d, J = 6.3 Hz, 3H), 3.11–3.14 (m, 1H), 3.25–3.40 (m, 4H), 3.55–3.62 (m, 1H), 3.75–3.82 (m, 1H), 4.31 (dd, J = 7.5, 14.1 Hz, 1H), 4.39 (d, J = 8.1 Hz, 0.6H), 4.43 (d, J = 8.1 Hz, 0.4H), 5.05–5.23 (m, 2H), 5.61–5.72 (m, 0.6H), 5.78–5.90 (m, 0.4H); ESIMS m/z: 238.1 [M + Na^+].

Treat of glucose (0.40 g, 2.2 mmol) with *P. dulcis* kernel meal (0.60 g) in benzyl alcohol/water (10 mL, 9:1, v/v) according to the general procedure gave benzyl β-D-glucopyranoside (**2l**) as white solid (0.304 g, 47%). $[\alpha]_D^{20}$ −55.5 (c 0.885, MeOH) ($[\alpha]_D^{20}$ −55.1, literature [18]); ^{1}H NMR (D_2O, ppm), δ/ppm: 3.26 (dd, J = 8.7, 8.4 Hz, 1H), 3.33 (m, 3H), 3.67 (dd, J = 5.6, 12.4 Hz, 1H), 3.87 (dd, J = 1.9, 12.4 Hz, 1H), 4.46 (d, J = 8.0 Hz, 1H), 4.69 (d, J = 11.6 Hz, 1H), 4.88 (d, J = 11.6 Hz, 1H), 7.34 (m, 5H); ^{13}C NMR (75 MHz, DMSO), δ/ppm: 61.2 (C-6), 69.4 (C-4), 70.2 (C-1′), 73.5 (C-2), 76.8 (C-3 or C-5), 76.9 (C-3 or C-5), 102.1 (C-1), 127.2, 127.5, 128.0, 138.0 (C-aryl); ESIMS m/z: 293.0 [M + Na^+].

Treat of glucose (0.54 g, 3 mmol) with *M. pumila* seed meal (0.60 g) in 2-chroloethanol/dioxane/water (10 mL, 3:6:1, v/v/v) according to the general procedure gave 2-chroloethyl β-D-glucopyranoside (**2m**) as clear syrup (0.093 g, 12%). $[\alpha]_D^{20}$ −25.2 (c 2.9, CH_3OH) ($[\alpha]_D^{20}$ −26.3, literature [32]); ^{1}H NMR (300 MHz, D_2O), δ/ppm: 3.17–3.25 (m, 1H), 3.18–3.42 (m, 3H), 3.55–3.69 (m, 3H), 3.79–3.90 (m, 2H), 4.02–4.09 (m, 1H), 4.43 (d, J = 7.8 Hz, 1H); ESIMS m/z: 264.9 [M + Na^+].

Treat of glucose (0.54 g, 3 mmol) and 4-hydroxyphenylethanol (4.9 g, 12 mmol) with *P. dulcis* kernel meal (0.60 g) in dioxane-water (10 mL, 9:1, v/v) according to the general procedure gave 4-hydroxyphenylethyl β-D-glucopyranoside (**2n**) as white solid (0.126 g, 14%). $[\alpha]_D^{20}$ −30.1 (c 0.50, CH_3OH) ($[\alpha]_D^{20}$ −28.4, literature [16]); ^{1}H NMR (300 MHz, CD_3OD), δ/ppm: 2.76 (m, 2H), 3.11 (m, 1H), 3.24 (m, 3H), 3.55 (dd, J = 5.8, 12.4 Hz, 1H), 3.74 (dd, J = 3.0, 7.0 Hz, 1H), 3.78 (dd, J = 1.9, 12.4 Hz, 1H), 3.96 (m, 1H), 4.34 (d, J = 8.0 Hz, 1H), 6.75 (d, J = 8.5 Hz, 1H), 7.11 (d, J = 8.5 Hz, 1H); ESIMS m/z: 323.15 [M + Na^+].

Treat of glucose (0.54 g, 3 mmol) and (E)-3-phenylprop-2-en-1-ol (4.8 g, 36 mmol) with *P. dulcis* kernel meal (0.60 g) in *tert*-butyl alcohol/water (10 mL, 9:1, v/v) according to the general procedure gave 3-phenylprop-2-enyl β-D-glucopyranoside (**2o**) as white solid (0.123 g, 14%); $[\alpha]_D^{20}$ −46.5 (c 0.50, CH_3OH) ($[\alpha]_D^{20}$ −48.8, literature [16]); ^{1}H NMR (300 MHz, CD_3OD), δ/ppm: 3.21–3.40 (m, 2H), 3.68 (dd, J = 5.4, 11.7 Hz, 1H), 3.88 (d, J = 12 Hz, 1H), 4.28–4.34 (m, 1H), 4.36 (d, J = 7.8 Hz, 1H), 4.50–4.56 (m, 1H), 6.32–6.41 (m, 1H), 6.67 (d, J = 15.9 Hz, 1H), 7.18–7.42 (m, 5H); ^{13}C NMR (75 MHz, CD_3OD), δ/ppm: 61.4, 69.4, 70.3, 73.7, 76.6, 76.7, 101.9, 125.3, 126.2, 127.4, 128.2, 132.4, 136.8; ESIMS m/z: 319.20 [M + Na^+].

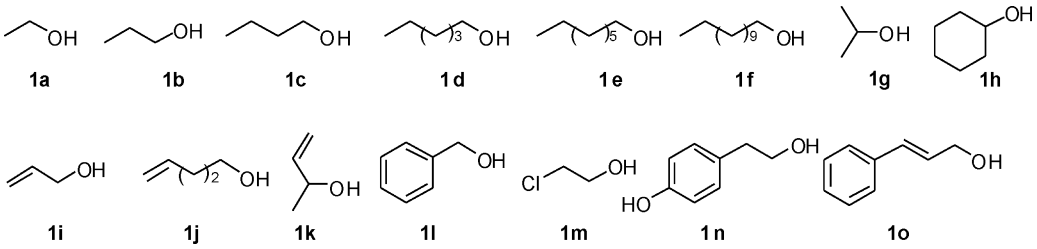
3. Results and discussion

3.1. Synthesis of alkyl β-glucosides using P. dulcis kernel meal

The *P. dulcis* (almond) kernel was soaked in distilled water for 2 h, peeled, air-dried, and then powdered in cold ethyl acetate with a homogenizer. The powder was defatted by further three washes with ethyl acetate, and two washes with acetone. This *P. dulcis* kernel meal was obtained as a white powder with 31.3 U/g hydrolysis activity and could be stored under 4 °C for 12 months without any detectable loss of activity.

The investigation for the reactivity of the obtained *P. dulcis* kernel meal was carried out via alkyl glucosidation with various alcohols. The *P. dulcis* kernel meal (60 mg or 1.9 U/mL) was added to a mixture of glucose and alcohol containing 10% (v/v) of water. The reaction mixtures were stirred for 48–72 h at 50 °C, and then filtered. The filtrates were concentrated, and the residues were subjected to silica gel flash column chromatography to give pure glucoisdes **2**. The obtained glucosides were all in β form and anomerically pure. The results were summarized in Table 1. The water content of the mixture was determined on the basis of our earlier results [33,34]. For comparison, a control experiment was also conducted using glucosidase from Sigma (G-0395, 5 mg, or 29.5 U/mL, the amount of glucosidase added was chosen according to literatures [18,19], whose hydrolysis

Table 1
Synthesis of alkyl β-glucosides using *P. dulcis* kernel meal



Entry	Substrate	[Glucose]$_0$ (M)	Time (h)	Yield 1[a] (%)	Yield 2[b] (%)
1	**1a**	0.50	72	45	42
2	**1b**	0.50	72	52	43
3	**1c**	0.28	72	39	38
4[c]	**1d**	0.30	72	13	12
5[c]	**1e**	0.28	60	12	11
6[c]	**1f**	0.30	72	0	0
7	**1g**	0.22	48	38	39
8	**1h**	0.30	72	10	11
9	**1i**	0.30	48	49	55
10	**1j**	0.30	72	45	47
11[d]	**1k**	0.30	72	25	27
12	**1l**	0.22	72	51	40
13[e]	**1m**	0.30	48	12	0
14[e]	**1n**	0.30	48	13	14
15[f]	**1o**	0.30	48	14	19

[a] Isolation yield, using 60 mg (1.9 U) of home-made acetone powder of *P. dulcis* kernel meal per mL of reaction mixture.
[b] Isolation yield, using 5 mg (29.5 U) of commercially supplied glucosidase preparation from almond (Sigma, G-0395) per mL of reaction mixture.
[c] 50% (v/v) CH_3CN was added as cosolvent.
[d] The product was slightly in favour of the (*R*)-isomer (*R*/*S* = 3/2, according to ^{1}H NMR spectrum).
[e] Dioxane was added as cosolvent.
[f] *tert*-Butyl alcohol was added as cosolvent.

activity was 16 times as that of *P. dulcis* kernel meal) as a catalyst (Table 1, yield 2) under the same condition.

As shown in Table 1, the yields of the glucosides obtained with the *P. dulcis* kernel meal made in-house were comparable with those of controls, although the hydrolysis activity of *P. dulcis* kernel meal is only 6.5% of the commercial one [18,19]. It was suggested that the glucosidase in whole cells (meal) performed better than that in free form (commercially available one). It was also shown that the catalytic performance of the *P. dulcis* kernel meal was very similar to that of the commercially available glucosidase preparation.

The *P. dulcis* kernel meal could tolerate with up to 30% (v/v) of 2-chlorethanol **1m** (Table 1, entry 13), and producing the corresponding β-glucoside in moderate yield, while the commercially available almond β-glucosidase afforded no product under the same condition. This result suggested that the glucosidase in the crude *P. dulcis* kernel meal is more robust than the commercially available pure enzyme. It might be explained by the fact that the glucosidase in whole cells was stabilized by other proteins in the almond and therefore kept in the originally natural manner.

3.2. Repeated use of *P. dulcis* kernel meal in the enzymatic synthesis of alkyl β-D-glucoside

To stabilize and recruit the glucosidase used in reverse hydrolysis, the enzyme is usually immobilized on an inert material such as XAD-4 [35,36] or polyacryamide-type bead [37,38]. It was found that the powder of *P. dulcis* kernel meal as a crude form of the β-glucosidase could be recycled for re-use by simple filtration, without any need to purify and immobilize the enzyme. The re-use potential of *P. dulcis* kernel meal was examined in alcohol-water monophase system. As indicated in Table 2, each run of biotransformation resulted in some loss of activity; however, after six reaction cycles (each for 48 h) at 50 °C, more than one-half of the initial activity

Table 2
Repeated use of *P. dulcis* kernel meal in the enzymatic synthesis of alkyl β-D-glucoside

Run	Substrate	[Glucose]$_0$ (M)	Yield[a] (%)
1	**1g**	0.22	37
2	**1g**	0.22	34
3	**1g**	0.22	30
4	**1g**	0.22	28
5	**1g**	0.22	23
6	**1g**	0.22	21
1	**1i**	0.30	49
2	**1i**	0.30	36
3	**1i**	0.30	32
4	**1i**	0.30	28

[a] Isolation yield. The reaction was conducted under 50 °C for 48 h. After the reaction mixture was filtered, the retrieved *P. dulcis* kernel meal was washed with corresponding alcohol and re-used immediately.

Table 3
The β-glucosidase activity and protein content of several fruit seed meals

Enzyme source (100 mg)	Total activity (U)	Total soluble protein[a] (mg)	Specific activity (U/mg)
Prunus dulcis kernel	3.13	21.54	0.15
Malus pumila seed	3.36	3.36	1.00
Prunus persica kernel	2.15	11.06	0.19
Prunus armeniaca kernel	1.72	3.63	0.47
Eriobotrya japonica kernel	0.10	0.30	0.33

[a] Protein was determined according to Bradford [26] using ovoalbumin as a standard.

Table 4
Synthesis of alkyl β-glucosides using fruit seed meal

Entry	Substrate	$[Glucose]_0$ (M)	Time (h)	Yield[a] (%) *Malus pumila*	Yield[a] (%) *Prunus persica*	Yield[a] (%) *Prunus armeniaca*	Yield[a] (%) *Eriobotrya japonica*
1	**1a**	0.50	72	72	64	36	35
2	**1b**	0.50	72	60	52	52	32
3	**1c**	0.28	72	44	46	39	22
4[b]	**1d**	0.30	72	15	12	13	6
5[b]	**1e**	0.30	72	12	12	12	0
6[b]	**1f**	0.30	72	6	3	0	0
7	**1g**	0.22	48	29	29	29	21
8	**1h**	0.30	72	7	10	10	n.d.
9	**1i**	0.30	48	33	67	45	22
10	**1l**	0.22	72	25	47	47	23
11[c]	**1o**	0.30	48	29	18	12	/

[a] Isolation yield. Conditions were not fully optimized; all glucosides obtained are in β form and anomerically pure; The reactions were conducted at 50 °C, each with 60 mg fruit seed meal per mL of reaction mixture.
[b] 50% (v/v) CH_3CN was added as cosolvent.
[c] *tert*-Butyl alcohol was added as cosolvent.

was still retained when isopropyl alcohol **1g** was used as the substrate.

3.3. Synthesis of alkyl β-glucosides using different fruits kernel meal

As indicated in Table 1, the substrate scope of the *P. dulcis* glucosidase was relatively broad, capable of accepting alkyl, alkenyl and aryl alkyl alcohols except long chain alkyl ($\geq C_{12}$) alcohols. To find new catalysts having better catalytic activity or different aglycone specificity from the almond glucosidase, some popular fruits, including *P. persica* L. var. *scleropersica* (Reich) Yü et Lu (Peach), *P. armeniaca* Lam. var. *ansu* (Maxim.) Yü et Lu (red apricot), *M. pumila* Mill (apple), and *E. japonica* (Thund.) Lindl. (loquat), were tested as potential sources of the glucosidase biocatalyst using a similar procedure to the *P. dulcis*, and their β-glucosidase activities were assayed by measuring the release of *p*-nitrophenol from (*p*-nitrophenyl β-D-glucopyranoside), as listed in Table 3.

In further investigation and comparison of the glucosidase from four different sources, those meals were applied to the glucosidation of various alcohols. The results were represented in Table 4. In consistency with the results in Table 3, *P. persica* and *M. pumila* enzymes, which had higher hydrolysis activities, achieved better results in most cases. Furthermore, the *M. pumila* glucosidase can accept dodecanol **1f** (Table 4, entry 6) as substrate, which was not recognized by almond glucosidase. This indicated that the *M. pumila* glucosidase had a different specificity toward aglycones and could be good complement to the almond enzyme. In contrast, *P. armeniaca* kernel meal showed little difference from the *P. dulcis* kernel meal.

Salidroside **2n** and Rosin **2o**, isolated from *Rhodiola sachalinensis*, have been shown to possess the medical functions such as resisting anoxia, microwave radiation and fatigue [39–41]. Salidroside **2n** was synthesized in 28% yield, while Rosin **2o** in 29% yield, using *M. pumila* seed meal. However, less than 15% yield was achieved using almond meal as catalyst under the same condition. Also, *tert*-butyl alcohol was found to be the most suitable cosolvent for both of the reactions.

4. Summary

We have developed some novel, cheap, and green biocatalysts for the facile synthesis of various alkyl β-D-glucopyranosides. Those easily available biocatalysts enable large-scale preparation of physiologically active β-D-glucopyranosides. The application in a column-type reactor for continuous production of salidroside **2n** is currently in progress.

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