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α-GLUCOSYLATION OF FLAVONOIDS: DIVERSITY OF ACCEPTORS AND PRODUCTS

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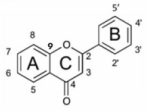
PURPOSE OF THE ABSTRACT
For many years phenolic compounds have had a strong success in the cosmetic industry due to their antioxidant, lightening, anti-inflammatory and UV-protection properties [1]. They are extracted from plants and some pure molecules like Arbutin, Hesperidin, Resveratrol, and more recently Bakuchiol, acquired a strong reputation.
Flavonoids, including Hesperidin, are a subfamily of polyphenols, with a C6-C3-C6 carbon skeleton composed of two phenyl rings (A and B) and a heterocyclic ring (C) (formation of ring C is not achieved in the case of dihydrochalcones) as seen in Figure 1 [2]. This structure makes flavonoids have low water solubility and low stability depending on substituents of ring A and B.
Glucosylation, the transfer of a glucose moiety from a donor to an acceptor by enzymatic reaction, is a way to increase water solubility and stability of phenolic compounds. In the 1990's Hayashibara developed industrial processes for the alpha-glucosylation of Hesperidin, Rutin and Naringin using Cyclodextrin glucanotransferase (CGTase) that usually works to convert starch and dextrins into cyclodextrins. The three flavonoids are natural glucosides and are recognized as substrate of the CGTase as they are structurally similar to its natural acceptors. While enzymatic glycosylation requires a specific recognition of substrate by the enzyme [3] an exhaustive review of the literature revealed that CGTases are able to transfer one or several glucose units on phenolic aglycones. As example CGTase from Thermoanaerobacter sp. can modify the following compounds at very variable molar yield (%): Kaempferol (15%), Pterostilbene (3%), Epigallocatechin gallate (65%), Resveratrol (50%), Hesperetin (4%) [4, 5, 6, 7, 8]. It appeared that conducting a study on the specificity of a CGTase to a large panel of phenolic compounds would be of great interest to understand how the structure of acceptor could influence the efficacy of conversion.

Thirty-one flavonoids, with varied chemical structures, were tested through glucosylation by CGTase from Thermoanaerobacter sp. As expected, natural glycosides were more prone to glucosylation and we found 7 aglycones exhibiting conversion yield above 45% (Figure 1). No absolute structural feature was identified as a predictive criterion for the success of conversion. However, molecular characteristics seemed to have a positive impact: the presence of glycosyl (Phloridzin vs Phloretin, Rutin vs Quercetin, Naringin vs Naringenin), the presence of glucuronyl (Baicalin vs Baicalein), the absence of methylation (Luteolin vs Hesperetin) and the hydroxylation in

position 3 (Myricetin vs Tricetin, Taxifolin vs Eriodictyol).
Then a comparison of epigallocatechin gallate (EGCG) alpha-glucosylation with two enzymes was conducted: Thermoanaerobacter sp. CGTase using alpha-cyclodextrin as donor and Dextransucrase (DS) from Leuconostoc mesenteroides NRRL B512F, using sucrose as donor [9,10]. Reaction medium was purified and fractionated by centrifugal partition chromatography to separate the different glucosides. Both enzymes mainly produced a diversity of glucosides of which the major one was a monoglucoside. NMR characterization of EGCG-G1 revealed that both enzymes generate two different structures: DS was more prone to create Epigallocatechin gallate 4'-O-glucoside as reported by [11], while CGTase mainly converts EGCG onto Epigallocatechin gallate 3'-O-glucoside as reported by [6]. Morevover, CGTase produced a diversity of glucosides where DS specifically synthesized mono- and diglucoside of EGCG (Figure 2).

Our work provides new insights in the broad diversity of acceptors of CGTase from Thermoanaerobacter sp. It also underlines the interest of glucosylation with alpha-transglucosidases that efficiently and specifically modify acceptors from cost-efficient and renewable material.

FIGURES

Molecule	% of conversion into alpha-glucoside	OH position	*O*-glycosyl / *O*-glucuronyl* position	OCH_3 position
				
Apigenin	0	5, 7, 4'	-	-
Chrysin	0	5, 7	-	-
Diosmetin	0	5, 7, 3'	-	4'
Diosmin	0	5, 3'	7	4'
Hesperetin	0	5, 7, 3'	-	4'
Baicalein	5	5, 6, 7	-	-
Tricetin	8	5, 7, 3', 4', 5'	-	-
Gossypetin	10	3, 5, 7, 8, 3', 4'	-	-
Naringenin	14	5, 7, 4'	-	-
Luteolin	18	5, 7, 3', 4'	-	-
Phloretin (dihydrochalcone)	26	5, 7, 9, 4'	-	-
Quercetin	28	3, 5, 7, 3', 4'	-	-
Fisetin	32	3, 7, 3', 4'	-	-
Hesperidin	36	5, 3'	7	4'
Neohesperidin dihydrochalcone	44	5, 9, 3'	7	4'
Naringin dihydrochalcone	45	5, 9, 3'	7	4'
Eriodictyol	47	5, 7, 3', 4'	-	-
Morin	47	3, 5, 7, 2', 4'	-	-
Epigallocatechin gallate	50	5, 7, 3', 4', 5'	-	-
Robinetin	59	3, 7, 3', 4', 5'	-	-
Quercetagetin	60	3, 5, 6, 7, 3', 4'	-	-
Baicalin	60	5, 6	7*	-
Rutin	62	5, 7, 4', 3'	3	-
Myricetin	65	3, 5, 7, 3', 4', 5'	-	-
Taxifolin	66	3, 5, 7, 3', 4'	-	-
Naringin	68	5, 4'	7	-
Isoquercitrin	75	5, 7, 4', 3'	3	-
Scutellarin	77	5, 6, 4'	7*	
Icariin	80	5	3, 7	4'
Neohesperidin	81	5, 3'	7	4'
Phloridzin (dihydrochalcone)	82	7, 9, 4'	5	-

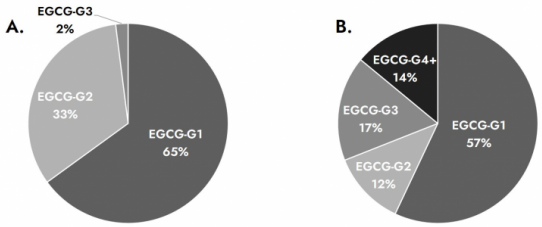


FIGURE 1

Characteristics of 31 flavonoids involved in transglucosylation with CGTase from Thermoanaerobacter sp.

The table reports the molar conversion yield of total alpha-glucosides and presents the main structural features of the molecules.

FIGURE 2

Distribution of Epigallocatechin gallate glucosides synthesized by Dextransucrase from Leuconostoc mesenteroides NRRL B512F (A) and Cyclodextrin glucanotransferase from Thermoanaerobacter sp (B) expressed as percentage of total glucosides.

Epigallocatechin glucosides are grouped under monoglucosides (EGCG-G1), diglucosides (EGCG-G2), triglucosides (EGCG-G3) and tetra- and polyglucosides (EGCG-4+).

KEYWORDS

Alpha-glucosylation | Flavonoids | Cyclodextrin glucanotransferase | Dextransucrase

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