Electronic Supplementary Material (ESI) for Green Chemistry.

Selective synthesis of citrus flavonoids prunin and naringenin using heterogeneized biocatalyst on graphene oxide

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Table S1. Elemental analysis of GO and RGO

Support	N%	C%	H%	S%
GO	0.0	45.6	2.5	1.5
RGO	0.0	79.3	1.3	0.0

Table S2. Amount of enzyme immobilized (%) of on GO and RGO in function of time

Time (h)	GO (%)	RGO (%)
0	0	0
3	66	(n.d.)
24	100	65

Table S3. Effect of the ionic force in the protein desorption

pH immobilization	[a]Desorbed Protein (0.5 M NaCl)	[a]Desorbed Protein (1 M NaCl)	%Total desorbed Protein
4.5	0.8	3.2	2.7
7	0	0	0
10	0	0	0

[a] (μg enzyme/mL)

Table S4. Comparative results of the long term stability and production capacity of naringinase immobilized on different materials

Support	Time (min)	Conversion (%)	Loss in conversion (n° Cycle)	TON[a]	Ref
Celite	15	35	31.6(2) 39.2(3)	0.22	1
K-carrageenan	120	70	35% (2) 62%(5)	2.00	2
Ca-alginate	30	70	50% (2) 66%(5)	1.80	3
PVA Hydrogel	1440	34	26.5% (2) 52.9%(6)	0.01	4
Chitin Crosslinking	36	60	Stable[d]	0.02	5
Reduced graphene	60	90	7.61%(10) 45.66%(15)	0.62[b]	6
Woodchips	60	76	Stable(7)[c]	1.09	7
Alginate Calcium	1440	56	50%(12)	-	8
Entrapped in cellulose triacetate films	30	31	Stable(20)[c]	0.14	9
Triacetate films	60	80	Stable[d]	0.08	10
Graphene oxide	30	99	Stable(10)[c] Stable(25)[c]	2.83(crude) 7.07(pure)	This work

[a] TON is defined as mg naringin hydrolyzed/mg immobilized naringinase. [b] mg isoquerticin hydrolyzed /mg immobilized naringinase [c] number of cycles [d] continuous process

Table S5. HPLC gradient

Time (minutes)	Gradient (%) water/ Acetonitrile
0-8	77/23
8-15	35/65
15-20	30/70
20-21	77/23
21-22	77/23
22-35	77/23

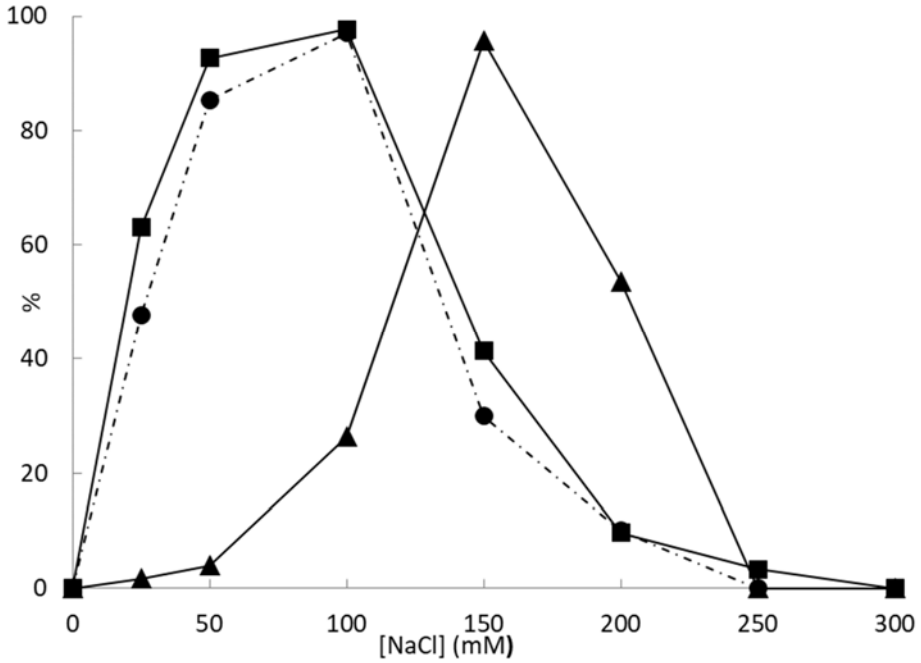

Figure S1. Desorption study in Naringinase purification (---●---) Activity for hydrolysis of Naringin, (—■—) Rhamnosidase Activity(using as substrate p-nitrophenyl-alpha-L-rhamnopyranoside), (—▲—) Glucosidase Activity (using as substrate p-nitrophenyl-β-D-glucoside)

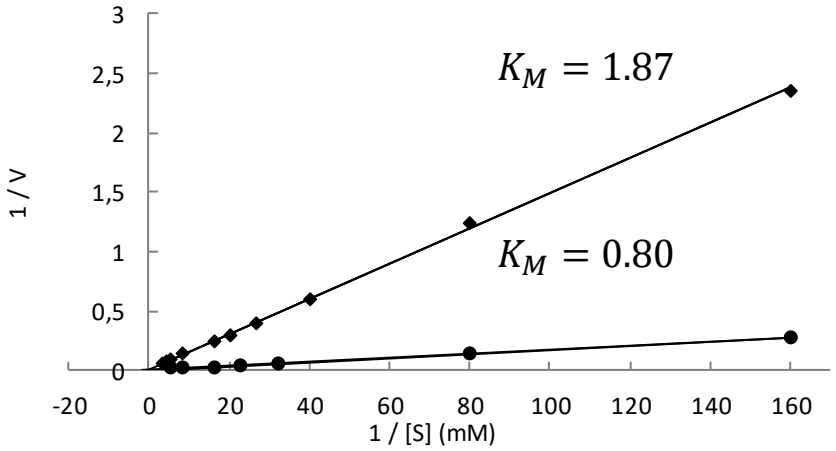

Figure S2(A). Lineweaver-Burk plots of crude naringinase in free and immobilized forms (—●—) GO-Crude, (—♦—) Free-Crude

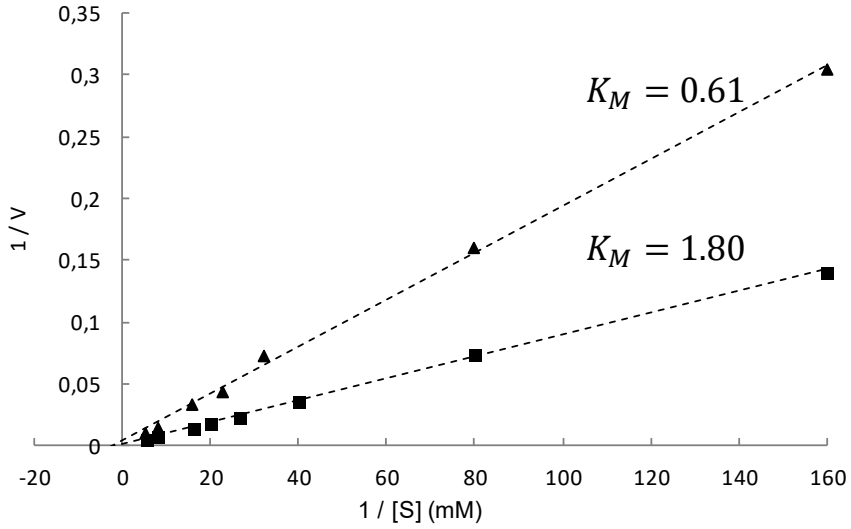

Figure S2(B). Lineweaver-Burk plots of pure naringinase in free and immobilized forms (--- ▲ ---) GO-Pure, (--- ■ ---) Free-Pure.

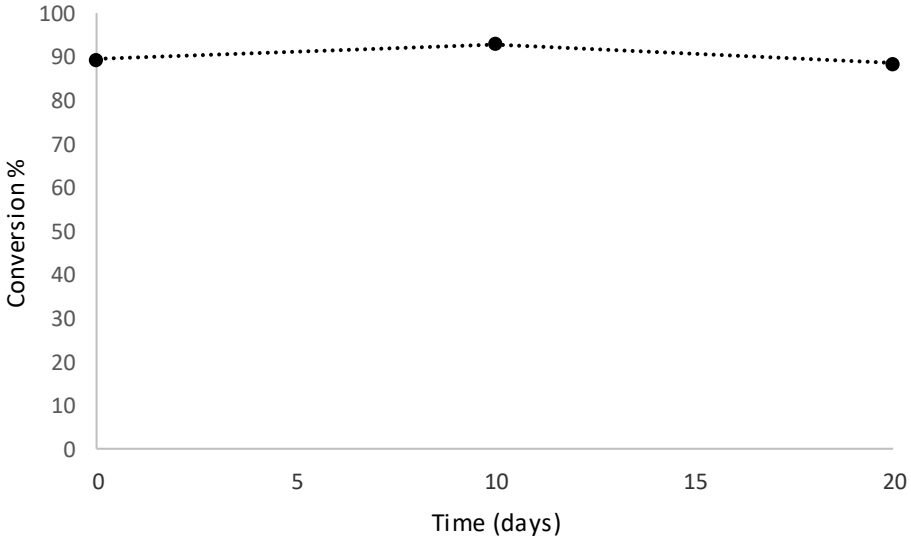

Figure S3. Conversion of naringin after different times of storage

References

1 G. Şekeroğlu, S. Fadiloğlu and F. Göğüş, *Eur. Food Res. Technol.*, 2006, **224**, 55.

2 I. A. C. Ribeiro and M. H. L. Ribeiro, *J. Mol. Catal. B Enzym.*, 2008, **51**, 10.

3 H. A. L. Pedro, A. J. Alfaia, J. Marques, H. J. Vila-Real, A. Calado and M. H. L. Ribeiro, *Enzyme Microb. Technol.*, 2007, **40**, 442.

4 M. D. Busto, V. Meza, N. Ortega and M. Perez-Mateos, *Food Chem.*, 2007, **104**, 1177.

5 H. Y. Tsen and S. Y. Tsai, *J. Ferment. Technol.*, 1988, **66**, 193.

6 A. Gong, C. T. Zhu, Y. Xu, F. Q. Wang, D. K. Tsabing, F. A. Wu and J. Wang, *Sci. Rep.*, 2017, **7**, 1.

7 M. Puri, H. Kaur and J. F. Kennedy, *J. Chem. Technol. Biotechnol.*, 2005, **80**, 1160.

8 M. Puri, S. S. Marwaha and R. M. Kothari, *Enzyme Microb. Technol.*, 1996, **18**, 281.

9 H. Y. Tsen and G. K. Yu, *J. Food Sci.*, 1991, **56**, 31.

10 H. Y. Tsen, S. Y. Tsai and G. K. Yu, *J. Ferment. Bioeng.*, 1989, **67**, 186.