SUPPLEMENTARY MATERIALS

Laccase activity as an essential factor in the oligomerization of rutin

Abel Muñiz-Mouro, Beatriz Gullón, Thelmo A. Lú-Chau, María Teresa Moreira, Juan M. Lema and Gemma Eibes*

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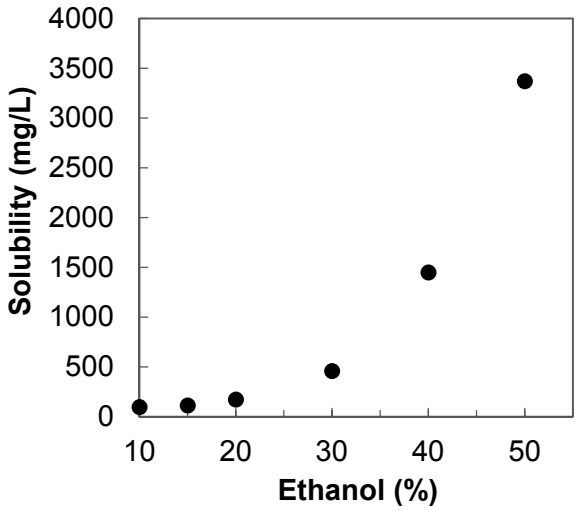

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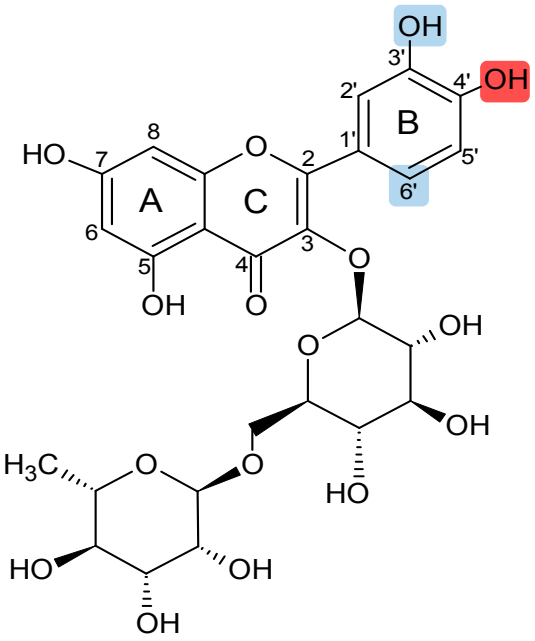

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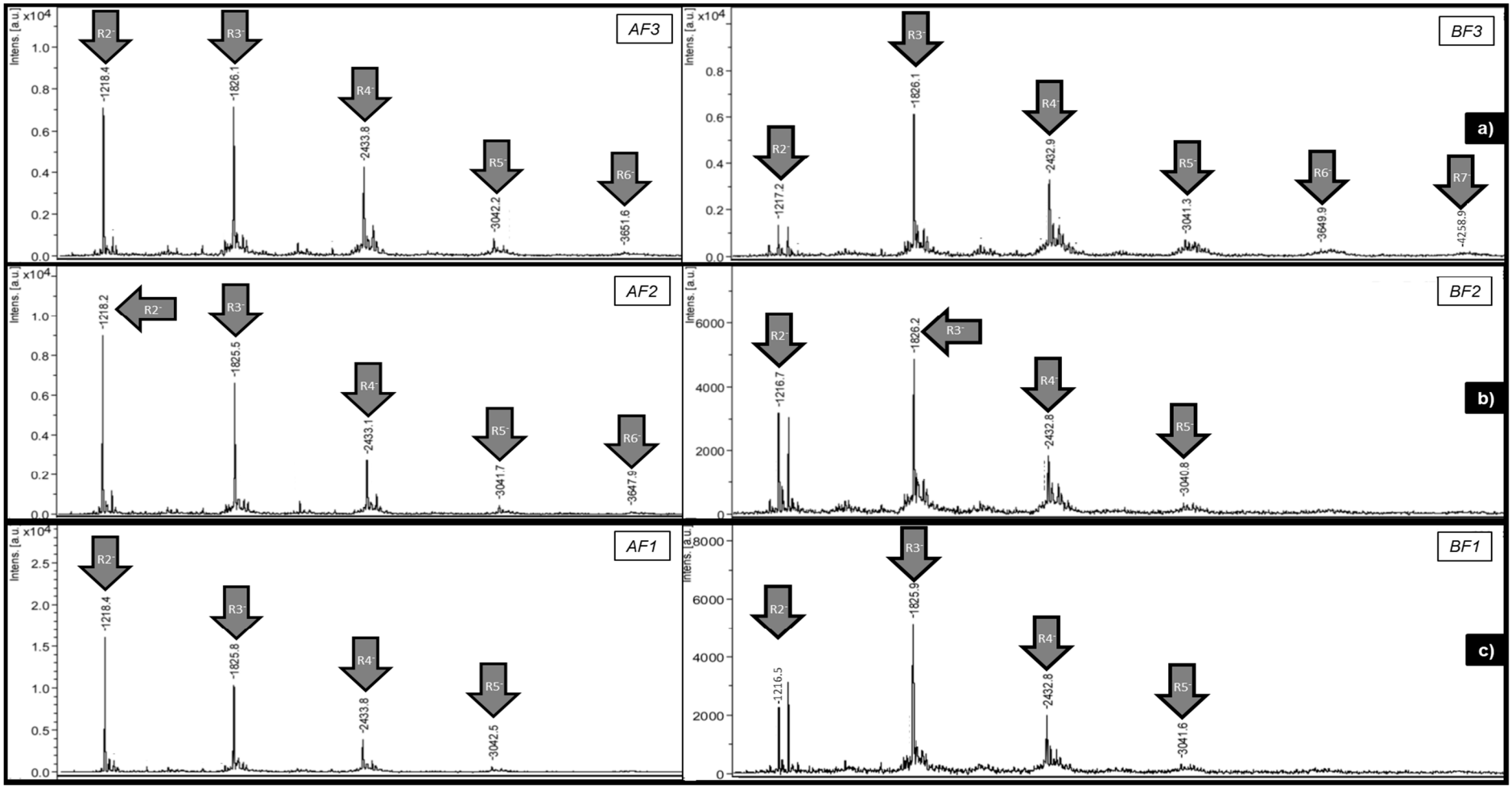

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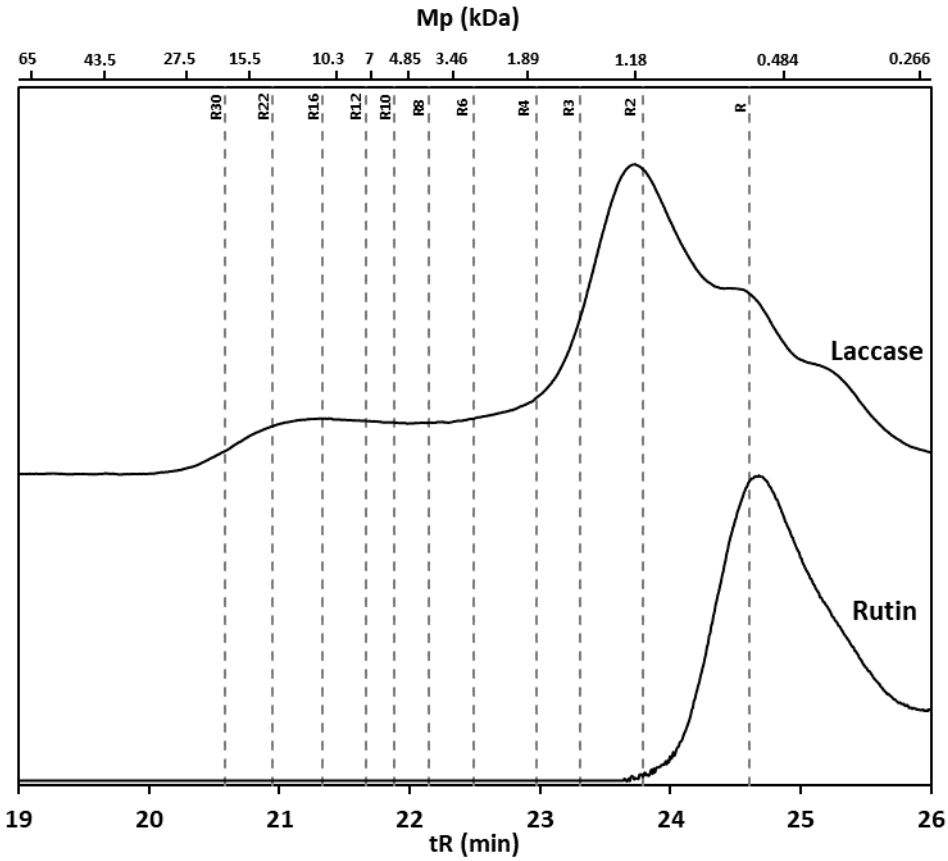

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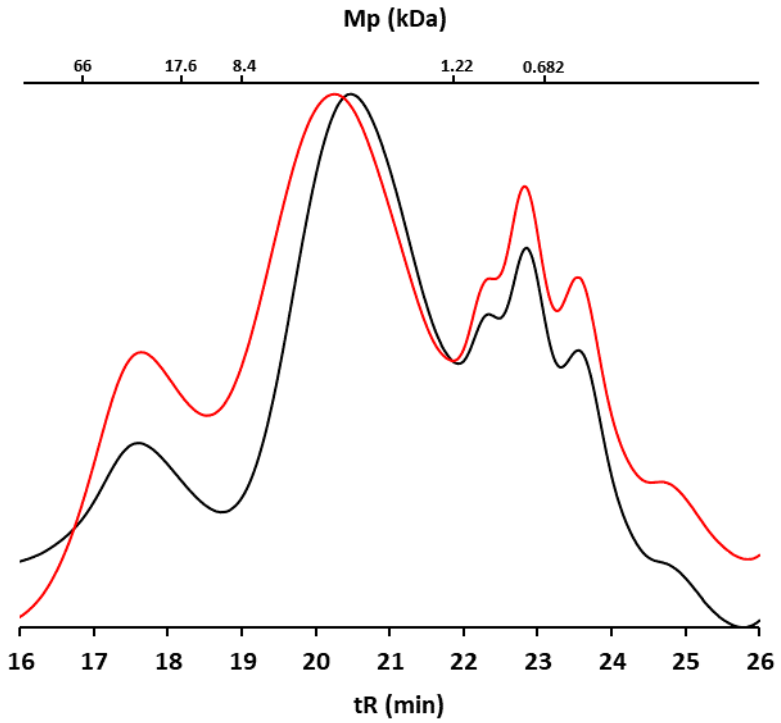

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Table S1. Total flavonoid content (TFC) in 10 g/L solutions of the different oligomer fractions in 50% MeOH:H2O and in supernatants of oversaturated (10 g/L) aqueous solutions.

	TFC of 10 g/L solutions (mg rutin eq./g sample)	
	MeOH:H_2O	**H_2O**
AF3	360.7 ± 18.2	243.9 ± 13.8
AF2	432.9 ± 14.8	253.2 ± 25.8
AF1	218.1 ± 18.8	47.9 ± 5.2
CA	624.6 ± 42.7	7.3 ± 0.1
BF3	154.4 ± 5.3	166.2 ± 2.5
BF2	20.4 ± 0.6	20.2 ± 1.1
BF1	*	*
CB	363.6 ± 10.4	5.5 ± 0.0

*: recovered lyophilized product was not sufficient to perform the assay. (A: 1,000 U/L laccase, B: 10,000 U/L laccase, C: control - F3 ≥ 10 kDa, F2 ⊂ (10-1 kDa], F1 < 1 kDa).

Table S2. MALDI-TOF results of the different fractions of rutin oligomers produced in reaction A and suggested compounds, detected as the deprotonated compounds.

AF3		AF2		AF1		Suggested compound*	Theoretical mass (Da)	[Δmass] (Da)		
Observed mass (Da)	Intensity	Observed mass (Da)	Intensity	Observed mass (Da)	Intensity			AF3	AF2	AF1
1189.7	135.9	1191.7	125.1	1192.2	170.2	$(R2\text{-}2xCH3)^-$	1190.0	0.29	1.76	2.20
						$(Q4)^-$	1201.9	0.69	1.16	0.92
1202.6~	339.2	1203.1	422.7	1202.8	504.1	$(R2\text{-}OH)^-$	1202.0	0.57	1.04	0.79
						$(R2\text{-}CH3)^-$	1204.0	1.40	0.93	1.18
1218.4	**7091.6**	**1218.2**	**8671.1**	**1218.4**	**16004.0**	**$(R2)^-$**	**1218.0**	**0.40**	**0.23**	**0.38**
1502.7	199.4	-	-	1504.2	205.0	$(Q5)^-$	1502.1	0.56	-	2.08
1518.2	506.4	1518.1	390.7	1517.5	589.5	$(R2+Q)^-$	1518.2	0.07	0.10	0.69
1532.6	210.8	1533.2	234.7	1534.5	363.0	$(R+2xI)^-$	1535.0	2.47	1.80	0.55
1680.1	479.9	1679.0	326.9	1680.2	449.0	$(R2+I)^-$	1680.8	0.70	1.76	0.60
1768.7	242.6	1768.2	172.2	1769.3	270.2	$(R3\text{-}3xCH3\text{-}OH)^-$	1768.4	0.23	0.20	0.84
1785.3	757.9	1784.9	484.1	1785.1	809.0	$(R3\text{-}3xCH3)^-$	1784.4	0.86	0.49	0.63
1798.1	601.0	1798.5	480.9	1797.3	591.7	$(R3\text{-}2xCH3)^-$	1798.5	0.35	0.00	1.20
1812.0	570.7	-	-	1811.7	629.4	$(R3\text{-}CH3)^-$	1812.5	0.50	-	0.75
1826.1	**7062.7**	**1825.5**	**6624.2**	**1825.8**	**10335.9**	**$(R3)^-$**	**1826.5**	**0.42**	**1.04**	**0.68**
2125.4	609.6	2124.5	613.0	2124.8	478.8	$(R3+Q)^-$	2126.7	1.32	2.20	1.98
2287.8	360.7	2286.4	225.6	2287.8	337.8	$(R3+I)^-$	2289.3	1.47	2.84	1.45
2376.9^	262.5	2375.2	237.6	2376.7	282.9	$(R4\text{-}3xCH3\text{-}OH)^-$	2376.9	0.06	1.71	0.23
						$(R4\text{-}4xCH3)^-$	2378.9	2.03	3.68	2.20
2393.1^	404.2	2392.1	197.4	2393.2	294.6	$(R4\text{-}2xCH3\text{-}OH)^-$	2391.0	2.10	1.16	2.21
						$(R4\text{-}3xCH3)^-$	2392.9	0.13	0.81	0.23
2404.7^	544.6	2403.0	303.0	2404.5	417.9	$(R4\text{-}CH3\text{-}OH)^-$	2405.0	0.27	1.98	0.51
						$(R4\text{-}2xCH3)^-$	2407.0	2.24	3.96	2.48
2422.4	530.7	2419.3	354.1	2420.5	593.6	$(R4\text{-}CH3)^-$	2421.0	1.45	1.66	0.47
2433.8	**4228.4**	**2433.1**	**2739.7**	**2433.8**	**3880.6**	**$(R4)^-$**	**2435.0**	**1.21**	**1.91**	**1.21**
2984.7^	170.0	2984.9	122.2	-	-	$(R5\text{-}3xCH3\text{-}OH)^-$	2985.4	0.69	0.51	-
						$(R5\text{-}4xCH3)^-$	2987.4	2.67	2.48	-
3001.4^	179.7	-	-	-	-	$(R5\text{-}2xCH3\text{-}OH)^-$	2999.5	1.89	-	-
						$(R5\text{-}3xCH3)^-$	3001.4	0.08	-	-
3014.5^	267.3	-	-	-	-	$(R5\text{-}CH3\text{-}OH)^-$	3013.5	1.05	-	-
						$(R5\text{-}2xCH3)^-$	3015.5	0.92	-	-
3042.2	**844.8**	**3041.7**	**434.8**	**3042.5**	**627.3**	**$(R5)^-$**	**3043.5**	**1.34**	**1.85**	**1.02**
3651.6	**190.9**	**3647.9**	**130.7**	**-**	**-**	**$(R6)^-$**	**3652.0**	**0.40**	**4.11**	**-**

*R/QX:Rutin/Quercetin oligomer with a degree of polymerization of X, Q: quercetin, I: isoquercetin. (A: 1000 U/L laccase - F3 ≥ 10 kDa, F2 ⊂ (10-1 kDa], F1 < 1 kDa). '+' and '-' symbols mean addition and subtraction of the following molecule part. Masses labelled with '^' have 2 suggested compounds, while ones marked with '~' have 3.

Table S3. MALDI-TOF results of the different fractions of rutin oligomers produced in reaction B and suggested compounds, detected as the deprotonated compounds.

BF3		BF2		BF1		Suggested compound*	Theoretical mass (Da)	[Δmass] (Da)		
Observed mass (Da)	Intensity	Observed mass (Da)	Intensity	Observed mass (Da)	Intensity			BF3	BF2	BF1
1217.2	**1323.1**	**1216.7**	**3167.1**	**1216.5**	**2273.2**	**(R2)$^-$**	**1218.0**	**0.78**	**1.28**	**1.56**
1501.8	206.1	1502.1	292.6	-	-	(Q5)$^-$	1502.1	0.31	0.05	-
1519.1	336.1	1516.6	396.3	1518.2	442.5	(R2+Q)$^-$	1518.2	0.89	1.68	0.08
1533.6	251.1	1532.0	424.0	-	-	*(R+2xI)*$^-$	1535.0	1.48	3.06	-
1680.6	320.6	1679.6	303.4	1679.4	397.0	(R2+I)$^-$	1680.8	0.22	1.22	1.38
1768.8	233.7	-	-	1771.3	310.6	(R3-3xCH3-OH)$^-$	1768.4	0.38	-	2.83
1784.6	633.2	1785.0	624.2	1783.3	545.8	(R3-3xCH3)$^-$	1784.4	0.20	0.54	1.10
1798.3	666.3	1797.2	608.2	1797.0	576.9	(R3-2xCH3)$^-$	1798.5	0.11	1.30	1.41
1812.3	590.1	1813.1	545.6	1810.9	547.2	(R3-CH3)$^-$	1812.5	0.15	0.65	1.58
1826.1	**6141.9**	**1826.2**	**4696.6**	**1825.9**	**5150.3**	**(R3)$^-$**	**1826.5**	**0.37**	**0.33**	**0.58**
2124.2	450.9	2124.3	362.2	2124.3	2124.3	(R3+Q)$^-$	2126.7	2.53	2.42	2.45
-	-	-	-	-	-	(R3+I)$^-$	2289.3	-	-	-
2377.3^	311.8	-	-	-	-	(R4-3xCH3-OH)$^-$	2376.9	0.31	-	-
						(R4-4xCH3)$^-$	2378.9	1.66	-	-
2391.7^	454.0	2391.6	304.5	2391.3	313.7	(R4-2xCH3-OH)$^-$	2391.0	0.71	0.65	0.35
						(R4-3xCH3)$^-$	2392.9	1.26	1.32	1.63
2405.8^	497.5	2404.7	418.5	2404.2	319.7	(R4-CH3-OH)$^-$	2405.0	0.80	0.34	0.74
						(R4-2xCH3)$^-$	2407.0	1.17	2.31	2.72
2421.3	636.7	2420.2	451.1	2420.5	478.0	(R4-CH3)$^-$	2421.0	0.36	0.80	0.53
2432.9	**3215.4**	**2432.8**	**1839.2**	**2432.8**	**2008.4**	**(R4)$^-$**	**2435.0**	**2.09**	**2.21**	**2.24**
3011.8^	368.3	-	-	-	-	(R5-CH3-OH)$^-$	3013.5	1.67	-	-
						(R5-2xCH3)$^-$	3015.5	3.64	-	-
-	-	3024.5	155.2	-	-	(R5-OH)$^-$	3027.5	-	2.99	-
3041.3	**724.0**	**3040.8**	**349.9**	**3041.6**	**326.4**	**(R5)$^-$**	**3043.5**	**2.24**	**2.69**	**1.87**
3649.9	**327.0**	**-**	**-**	**-**	**-**	**(R6)$^-$**	**3652.0**	**2.11**	**-**	**-**
4258.9	**149.1**	**-**	**-**	**-**	**-**	**(R7)$^-$**	**4260.5**	**1.58**	**-**	**-**

*R/QX:Rutin/Quercetin oligomer with a degree of polymerization of X, Q: quercetin, I: isoquercetin. (B: 10,000 U/L laccase - F3 ≥ 10 kDa, F2 ⊂ (10-1 kDa], F1 < 1 kDa). '+' and '-' symbols mean addition and subtraction of the following molecule part. Masses labelled with '^' have 2 suggested compounds, while ones marked with '~' have 3.

Table S4. Thermogravimetric analysis (TGA) main parameters for high molecular mass rutin oligomers obtained in reactions A and B, compared to control experiments. MRDT: maximum rate decomposition temperature.

	Decomposition intervals (ºC)	Mass loss (%)	MRDT (ºC)	Solid residue at 600ºC (%)
AF3	31.2 - 163.2	3.8	263.3	60.8
	163.2 – 600.0	35.2		
CA	32.2 - 165.5	3.1	263.3	62.9
	165.5 - 600.0	34.2		
BF3	30.0 - 151.4	8.6	263.3	25.7
	151.4 - 414.2	39.7		
	414.2 - 600.0	25.0		
CB	31.8 - 146.8	4.2	269.1	47.5
	146.8 - 600.0	48.0		

(A: 1,000 U/L lacasse, B: 10,000 U/L laccase, C: control - F3 ≥ 10 kDa).

Table S5. Summary of tested oligorutin properties.

Laccase dosage (U/L)	**1,000**				**10,000**			
Product fraction	**AF3**	**AF2**	**AF1**	**CA**	**BF3**	**BF2**	**BF1**	**CB**
Apparent aqueous solubility	++	++	+	-	+++	+++	*	-
AO activity								
FRAP	++	++	+	+++	-	--	--	+
CUPRAC	+++	++	+	+++	+	--	--	+
ABTS	+++	+++	+	+++	+	--	--	++
Xanthine oxidase inhibition	+++	+++	+	++	++	--	--	+
Thermal stability	+++	n.t.	n.t.	+++	-	n.t.	n.t.	+

*: recovered lyophilized product was not sufficient to perform the assay, **n.t.**: not tested. (A: 1,000 U/L laccase, B: 10,000 U/L laccase, C: Control - F3 ≥ 10 kDa, F2 ⊂ (10-1 kDa], F1 < 1 kDa).