



### **Supplementary material:**

# Flavonoids with Glutathione Antioxidant Synergy: Influence of Free Radicals Inflow

## 4 Igor Ilyasov, Vladimir Beloborodov, Daniil Antonov, Anna Dubrovskaya, Roman Terekhov, 5 Anastasiya Zhevlakova, Asiya Saydasheva, Vladimir Evteev and Irina Selivanova

Initial ABTS** concentration, $\mu M$	Decrease rate, µM/min							
	0–1 min	1–10 min	10–15 min	15–20 min	20–25 min	25–30 min		
33.3 (initial absorbance 0.50)	0.32±0.19	$0.17 \pm 0.04$	0.11±0.01	0.10±0.02	0.09±0.01	0.08±0.02		
50.0 (initial absorbance 0.75)	0.28±0.11	$0.20\pm0.05$	$0.12 \pm 0.04$	0.11±0.01	$0.09 \pm 0.01$	0.08±0.02		
66.7 (initial absorbance 1.00)	0.33±0.09	$0.27 \pm 0.04$	0.15±0.03	0.12±0.02	$0.09 \pm 0.01$	0.09±0.02		
133.3 (initial absorbance 2.00)	$0.51 \pm 0.10$	0.28±0.03	$0.15 \pm 0.05$	0.13±0.02	$0.09\pm0.02$	$0.08 \pm 0.03$		

#### 6 **Table 1S.** ABTS<sup>++</sup> self-bleaching at different initial absorbances

**Table 2S.** Influence of different initial ABTS<sup>++</sup> concentrations on the antioxidant mixture effect of
 quercetin–glutathione 1:5 in the decolorization assay

Quercetin–glutathione 1:5								
C₀(ABTS · +), μM	Traditionally calcul	ated	Webb's simulation					
133.00	-0.07±5.68	a	6.7±6.3	a				
66.67	-7.15±3.20	d	4.8±0.3	b				
50.00	-10.28±2.16	b	10.2±2.8	с				
33.33	-6.26±6.47	a	8.7±5.9	a				

9 a-statistically insignificant, p > 0.05; b, c, d-statistically significant with p-values less than 0.05, 0.01 and 0.001, 10 and 0.001, 10

10 respectively.

12 quercetin–glutathione 1:5 combination in the decolorization assay

Quercetin-glutathione 1:5								
Addition order	Traditionally calculated mixture effect, %				Webb's simulation mixture effect, %			
ABTS•++ quercetin + (after 1 min) glutathione	-8.73	±	3.07	С	6.97	±	3.60	с
ABTS•+ + glutathione + (after 1 min) quercetin	-7.88	±	2.97	С	7.97	±	3.48	с

13 c – statistically significant with p-value less than 0.01

14

<sup>11</sup> **Table 3S.** Influence of different component addition orders on the antioxidant mixture effect of the



17

**Figure 1S.** Susceptibility to different initial ABTS<sup>•+</sup> concentrations of the antioxidant mixture effect in the decolorization assay: 133.3, 66.7 and 50  $\mu$ M, respectively;  $\lambda$  730 nm, pathlength 1 cm.

18 The ABTS<sup>++</sup> absorbance-time curves (**a**, **b**, and **c**) and absorbance loss ( $\Delta$ Abs) diagram (**d**) 19 demonstrates the experimentally derived results for individual components of combinations 20 (quercetin and glutathione), the combination of quercetin–glutathione 1:5 (experimental), and 21 theoretically calculated effect for combination obtained by simple summation (theoretical) or by 22 applying Webb's simulation (simulation by Webb).

23





**Figure 2S.** Susceptibility to different component addition orders of the antioxidant mixture effect in the decolorization assay;  $\lambda$  730 nm, pathlength 1 cm.

27 On the left (a and c): glutathione was mixed with ABTS<sup>++</sup> and then quercetin was added 1 min later. 28 On the right (**b** and **d**): quercetin was mixed with ABTS<sup>++</sup> and then glutathione was added 1 min later. 29 The ABTS<sup>•+</sup> absorbance loss ( $\Delta$ Abs) diagrams (**a**, **b**) and absorbance–time curves (**c**, **d**) demonstrate 30 the experimentally derived results for individual components: glutathione after 1 min incubation 31 (glutathione 1 min), quercetin after 1 min incubation (quercetin 1 min), and final results for quercetin, 32 glutathione (after 30 min) and quercetin-glutathione 1:5 (experimental, after 30 min), and 33 theoretically calculated effect for the combination obtained by simple summation (theoretical) or by 34 applying Webb's simulation (simulation by Webb).

#### Quercetin-glutathion 1:5





41 Figure 3S. The inhibition of ABTS<sup>++</sup> by flavonoids and glutathione.

- 42 On the left: absorbance decrease-time curves for taxifolin (a), quercetin (c), rutin (e), morin (g) and 43 glutathione (i).
- 44 On the right: inhibition percentage-glutathione concentration regressions at different time points for 45 taxifolin (b), quercetin (d), rutin (f), morin (h), and glutathione (j).  $\lambda$  730 nm, pathlength 1 cm.





49 50

52

**Figure 4S.** Antioxidant mixture effect of flavonoid–glutathione combinations at different ratios in the decolorization assay: taxifolin–glutathione ( $\mathbf{a}$ ,  $\mathbf{b}$ ), quercetin–glutathione ( $\mathbf{c}$ ,  $\mathbf{d}$ ), rutin–glutathione ( $\mathbf{e}$ ,  $\mathbf{f}$ ) and morin–glutathione ( $\mathbf{g}$ ,  $\mathbf{h}$ );  $\lambda$  730 nm, pathlength 1 cm, incubation time 30 min .

53 The ABTS<sup>+</sup> absorbance loss (ΔAbs) diagrams (a, c, e, g) and absorbance-time curves (b, d, f, h) 54 demonstrate the experimentally derived results for individual components (flavonoid and 55 glutathione), flavonoid–glutathione (experimental) and the theoretically calculated effect obtained by 56 simple summation (theoretical) or by applying Webb's simulation (simulation by Webb).

57



61

59

60

Figure 5S. Antioxidant mixture effect of flavonoid–glutathione combinations at different ratios in the
 lag-time assay: taxifolin–glutathione (a), quercetin–glutathione (b), rutin–glutathione (c) and morin–
 glutathione (d); λ 730 nm, pathlength 1 cm.

65The lag-time diagrams (a-d) demonstrate the experimentally derived results for individual66components (flavonoid and glutathione), flavonoid-glutathione (experimental) and the theoretically67calculated effect obtained by simple summation (theoretical).

Antioxidants 2020, 9, 695



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).