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# **Assessment of the Antioxidant Capacity of Commercial Coffee Using Conventional Optical and Chromatographic Methods and an Innovative Electrochemical DNA-Based Biosensor**

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**Table 1S.** Total antioxidant capacity of commercial coffee samples measured by a DNA-based biosensor and optical methods.

| Sample    | DNA-based biosensor           |  |                             | Optical methods               |                            |                             |
|-----------|-------------------------------|--|-----------------------------|-------------------------------|----------------------------|-----------------------------|
|           | OH <sup>•</sup> mg GAE/L      | H <sub>2</sub> O <sub>2</sub> mg GAE/L | TPC mg GAE/L                | TFC mg ECE/L                  | FRAP mg AAE/L              | DPPH mg TE/L                |
| <b>1</b>  | 448.4 ± 12.1 <sup>a,b,c</sup> | 227.9 ± 0.6 <sup>a</sup>               | 5611 ± 203 <sup>a,b,c</sup> | 2566 ± 167 <sup>a,b,c</sup>   | 4468 ± 132 <sup>a,b</sup>  | 24154 ± 4719 <sup>a,b</sup> |
| <b>2</b>  | 939.4 ± 220.5 <sup>b,d</sup>  | 1562 ± 34 <sup>b</sup>                 | 7343 ± 194 <sup>b</sup>     | 3280 ± 259 <sup>b,d</sup>     | 5062 ± 19 <sup>a</sup>     | 25811 ± 948 <sup>a,b</sup>  |
| <b>3</b>  | 1771 ± 135 <sup>e</sup>       | 1824 ± 126 <sup>b</sup>                | 4612 ± 146 <sup>a</sup>     | 1940 ± 143 <sup>a</sup>       | 3493 ± 14 <sup>c</sup>     | 16990 ± 643 <sup>a</sup>    |
| <b>4</b>  | 523.2 ± 52.2 <sup>a,b,c</sup> | 1060 ± 58 <sup>c</sup>                 | 6288 ± 760 <sup>a,b,c</sup> | 3162 ± 211 <sup>b,c,d</sup>   | 4702 ± 68 <sup>a</sup>     | 29540 ± 4029 <sup>b</sup>   |
| <b>5</b>  | 303.3 ± 75.3 <sup>a,c</sup>   | 1021 ± 65 <sup>c,d</sup>               | 5966 ± 133 <sup>a,b,c</sup> | 3588 ± 76 <sup>d</sup>        | 4933 ± 56 <sup>a</sup>     | 21308 ± 5036 <sup>a,b</sup> |
| <b>6</b>  | 1275 ± 269 <sup>d,e</sup>     | 1709 ± 99 <sup>b</sup>                 | 4670 ± 76 <sup>a</sup>      | 2300 ± 7 <sup>a,c</sup>       | 3683 ± 55 <sup>b,c</sup>   | 19964 ± 1843 <sup>a,b</sup> |
| <b>7</b>  | 155.9 ± 12.8 <sup>a</sup>     | 193.4 ± 10.8 <sup>a</sup>              | 5447 ± 98 <sup>a,c</sup>    | 2743 ± 114 <sup>a,b,c,d</sup> | 4279 ± 85 <sup>a,b,c</sup> | 22047 ± 473 <sup>a,b</sup>  |
| <b>8</b>  | 454.5 ± 27.9 <sup>a,b,c</sup> | 1187 ± 88 <sup>c</sup>                 | 5787 ± 272 <sup>a,b,c</sup> | 3304 ± 63 <sup>b,d</sup>      | 5161 ± 1 <sup>a</sup>      | 21942 ± 955 <sup>a,b</sup>  |
| <b>9</b>  | 559.5 ± 38.6 <sup>a,b,c</sup> | 2422 ± 20 <sup>e</sup>                 | 11807 ± 995 <sup>d</sup>    | 3504 ± 670 <sup>d</sup>       | 9942 ± 548 <sup>d</sup>    | 15725 ± 969 <sup>a</sup>    |
| <b>10</b> | 425.3 ± 13.4 <sup>a,c</sup>   | 2297 ± 54 <sup>e</sup>                 | 6449 ± 225 <sup>b,c</sup>   | 2909 ± 116 <sup>b,c,d</sup>   | 6248 ± 187 <sup>e</sup>    | 19837 ± 3343 <sup>a,b</sup> |
| <b>11</b> | 322.2 ± 76.5 <sup>a,c</sup>   | 335.0 ± 71.4 <sup>a</sup>              | 10149 ± 719 <sup>d,e</sup>  | 3091 ± 123 <sup>b,c,d</sup>   | 8163 ± 293 <sup>f</sup>    | 24921 ± 2594 <sup>a,b</sup> |
| <b>12</b> | 688.1 ± 200.5 <sup>b,c</sup>  | 699.4 ± 187.0 <sup>d</sup>             | 9296 ± 187 <sup>e</sup>     | 2894 ± 40 <sup>b,c,d</sup>    | 13805 ± 504 <sup>g</sup>   | 16954 ± 24 <sup>a</sup>     |

**Table 2S.** Phenolic compounds identified and quantified in coffee and coffee substitutes available in the Portuguese market; results, in mg/L of coffee, are expressed as mean  $\pm$  standard deviation of three replicates.

| Phenolic compound          | Retention time / min | Coffee 1          | Coffee 2             | Coffee 3              | Coffee 4               | Coffee 5                 | Coffee 6             | Coffee 7               | Coffee 8               | Coffee 9               | Coffee 10               | Coffee 11              | Coffee 12              |
|----------------------------|----------------------|-------------------|----------------------|-----------------------|------------------------|--------------------------|----------------------|------------------------|------------------------|------------------------|-------------------------|------------------------|------------------------|
| Gallic acid                | 5.618                | 20.4 $\pm$ 1.0b   | 360 $\pm$ 18a        | 42.5 $\pm$ 2.1<br>b,f | 236 $\pm$ 12d          | 61.1 $\pm$ 3.1e,f<br>,g  | 98.2 $\pm$ 4.9<br>c  | 71.2 $\pm$ 3.6e<br>,g  | 79.1 $\pm$ 4.0c,<br>e  | 51.8 $\pm$ 2.6f,<br>g  | 67.8 $\pm$ 3.4e,<br>f,g | 102 $\pm$ 5c           | 405 $\pm$ 20h          |
| Protocatechuic acid        | 9.935                | 179 $\pm$ 9b      | 360 $\pm$ 18a        | 528 $\pm$ 26d         | 466 $\pm$ 23c,d        | 651 $\pm$ 33f            | 695 $\pm$ 35f        | 347 $\pm$ 17a,<br>e    | 416 $\pm$ 21c          | 285 $\pm$ 14e          | 332 $\pm$ 17a,e         | 460 $\pm$ 23c          | 300 $\pm$ 15a,<br>e    |
| Neochlorogenic acid        | 10.219               | 209 $\pm$ 10b     | 474 $\pm$ 24a        | 406 $\pm$ 20c         | 396 $\pm$ 20c          | 514 $\pm$ 26a            | 618 $\pm$ 31e        | 340 $\pm$ 17c,d<br>,f  | 458 $\pm$ 23a,c        | 299 $\pm$ 15d          | 332 $\pm$ 17d,f         | 487 $\pm$ 24a          | 385 $\pm$ 19c,f        |
| (+)-Catechin               | 14.143               | 19.9 $\pm$ 1.0b   | 107 $\pm$ 5a         | 45.5 $\pm$ 2.3<br>b,f | 38.5 $\pm$ 1.9b        | 67.4 $\pm$ 3.4f,h        | 300 $\pm$ 15c        | 235 $\pm$ 12d,<br>g    | 214 $\pm$ 11d          | 161 $\pm$ 8e           | 248 $\pm$ 12g           | 302 $\pm$ 15c          | 76.8 $\pm$ 3.8h        |
| Caftaric acid              | 15.436               | ND                | ND                   | ND                    | ND                     | ND                       | ND                   | 11.1 $\pm$ 0.6b        | ND                     | ND                     | 11.7 $\pm$ 0.6b         | 14.0 $\pm$ 0.7a        | ND                     |
| Caffeine                   | 16.154               | 46.3 $\pm$ 2.3b   | 726 $\pm$ 36a,<br>e  | 643 $\pm$ 32a,<br>d   | 607 $\pm$ 30d          | 842 $\pm$ 42c            | 907 $\pm$ 45c        | 692 $\pm$ 35a,<br>d    | 822 $\pm$ 41c,e        | 637 $\pm$ 32a,d        | 254 $\pm$ 13f           | 838 $\pm$ 42c          | 453 $\pm$ 23g          |
| Chlorogenic acid           | 17.869               | 71.6 $\pm$ 3.6b   | 469 $\pm$ 23a        | 769 $\pm$ 38d         | 741 $\pm$ 37d          | 1003 $\pm$ 50g           | 1168 $\pm$ 58<br>f   | 566 $\pm$ 28a          | 835 $\pm$ 42c,d        | 319 $\pm$ 16e          | 20.4 $\pm$ 1.0b         | 885 $\pm$ 44c          | 52.3 $\pm$ 2.6b        |
| 4-O-caffeyolquinic acid    | 19.897               | 15.7 $\pm$ 0.8a,g | 40.9 $\pm$ 2.0<br>a  | 346 $\pm$ 17b         | 387 $\pm$ 19c          | 66.2 $\pm$ 3.3d          | 485 $\pm$ 24e        | 336 $\pm$ 17c,e        | 541 $\pm$ 27e          | 329 $\pm$ 16b,f        | 363 $\pm$ 18g           | 497 $\pm$ 25c,e        | 451 $\pm$ 23f          |
| Vanillic acid              | 20.748               | 80.9 $\pm$ 4.0a   | 524 $\pm$ 26b        | 30.0 $\pm$ 1.5<br>c   | 1.52 $\pm$ 0.08<br>d   | 1.91 $\pm$ 0.10c         | 2.47 $\pm$ 0.1<br>2c | 18.7 $\pm$ 0.9c        | 337 $\pm$ 17d          | 215 $\pm$ 11e          | 251 $\pm$ 13f           | 31.2 $\pm$ 1.6c        | 241 $\pm$ 12e,f        |
| Caffeic acid               | 21.224               | 11.4 $\pm$ 0.6b   | 41.3 $\pm$ 2.1<br>a  | 50.7 $\pm$ 2.5<br>e   | 98.0 $\pm$ 4.9d        | 47.9 $\pm$ 2.4e          | 61.0 $\pm$ 3.1f      | 47.9 $\pm$ 2.4c        | 90.2 $\pm$ 4.5c        | 49.0 $\pm$ 2.5d        | 61.0 $\pm$ 3.1d         | 72.4 $\pm$ 3.6g        | 9.84 $\pm$ 0.49<br>g   |
| Syringic acid              | 22.283               | <LOD              | <LOQ                 | 10.7 $\pm$ 0.5<br>b   | <LOQ                   | ND                       | 1.04 $\pm$ 0.0<br>5d | 5.25 $\pm$ 0.26<br>c   | 11.3 $\pm$ 0.6a,<br>b  | 5.37 $\pm$ 0.27<br>c   | 16.6 $\pm$ 0.8e         | 11.9 $\pm$ 0.6a        | 10.4 $\pm$ 0.5b        |
| (-)-Epicatechin            | 23.294               | <LOD              | <LOQ                 | 113 $\pm$ 6d          | 5.33 $\pm$ 0.27<br>b   | 13.2 $\pm$ 0.7c          | 14.7 $\pm$ 0.7<br>c  | 15.8 $\pm$ 0.8c        | 103 $\pm$ 5a           | 14.9 $\pm$ 0.7c        | 112 $\pm$ 6d            | 101 $\pm$ 5a           | 35.4 $\pm$ 1.8e        |
| p-Coumaric acid            | 33.758               | <LOQ              | 7.02 $\pm$ 0.3<br>5a | ND                    | ND                     | ND                       | ND                   | ND                     | 6.67 $\pm$ 0.33<br>a   | ND                     | 6.53 $\pm$ 0.33<br>a    | <LOQ                   | ND                     |
| trans-Ferulic acid         | 37.289               | 0.55 $\pm$ 0.03b  | 4.09 $\pm$ 0.2<br>0a | 14.7 $\pm$ 0.7<br>e   | 1.90 $\pm$ 0.09<br>c,d | 2.22 $\pm$ 0.14c<br>,d,f | 11.0 $\pm$ 0.6<br>g  | 3.04 $\pm$ 0.15<br>f   | 4.07 $\pm$ 0.20<br>a   | 1.43 $\pm$ 0.07<br>b,d | 10.7 $\pm$ 0.5g         | 2.71 $\pm$ 0.14<br>c,f | 3.86 $\pm$ 0.19<br>a   |
| Sinapic acid               | 37.662               | 19.7 $\pm$ 1.0b   | 68.0 $\pm$ 3.4<br>a  | 46.2 $\pm$ 2.3<br>c,d | 46.7 $\pm$ 2.3c,<br>d  | 28.4 $\pm$ 1.4e          | 41.3 $\pm$ 2.1<br>c  | 25.4 $\pm$ 1.4b<br>,e  | 49.7 $\pm$ 2.5d        | 25.3 $\pm$ 1.3b        | 29.1 $\pm$ 1.5e         | 43.1 $\pm$ 2.2c        | 8.28 $\pm$ 0.41<br>f   |
| trans-polydatin            | 39.182               | 1.59 $\pm$ 0.08b  | 11.2 $\pm$ 0.6<br>a  | 24.6 $\pm$ 1.2<br>d   | 13.4 $\pm$ 0.7a        | 13.0 $\pm$ 0.6a          | 40.4 $\pm$ 2.0<br>e  | 11.8 $\pm$ 0.6a        | 18.3 $\pm$ 0.9c        | 12.4 $\pm$ 0.6a        | 26.8 $\pm$ 1.3d         | 18.1 $\pm$ 0.9c        | 7.28 $\pm$ 0.36<br>f   |
| 3,5-di-caffeoylquinic acid | 50.127               | <LOD              | 45.8 $\pm$ 2.3<br>a  | ND                    | 39.3 $\pm$ 2.0b        | ND                       | ND                   | ND                     | 99.6 $\pm$ 5.0c        | 17.9 $\pm$ 0.9d        | 33.9 $\pm$ 1.7b         | 38.7 $\pm$ 1.9b        | ND                     |
| Quercetin-3-O-galactoside  | 52.177               | 8.11 $\pm$ 0.41b  | 27.6 $\pm$ 1.4<br>a  | 38.4 $\pm$ 1.9f       | 23.5 $\pm$ 1.2d        | 3.68 $\pm$ 0.18c         | 33.4 $\pm$ 1.7<br>g  | 4.88 $\pm$ 0.24<br>c,e | 7.14 $\pm$ 0.36<br>b,e | 6.58 $\pm$ 0.33<br>b,e | 19.9 $\pm$ 1.0h         | 5.17 $\pm$ 0.26<br>c,e | 4.85 $\pm$ 0.24<br>c,e |

|                                      |        |                  |                  |                 |                |            |                 |                |                  |                |                  |                  |                |
|--------------------------------------|--------|------------------|------------------|-----------------|----------------|------------|-----------------|----------------|------------------|----------------|------------------|------------------|----------------|
| <b>Quercetin-3-O-glucopyranoside</b> | 52.735 | <LOD             | 14.3±0.7<br>a    | 8.61±0.4<br>3e  | 13.0±0.6c      | ND         | 6.65±0.3<br>3f  | 1.83±0.09<br>b | 4.57±0.23<br>d   | 2.04±0.10<br>b | ND               | 2.09±0.10<br>b   | ND             |
| <b>Rutin</b>                         | 53.284 | 0.71±0.04b,<br>e | <LOD             | 1.21±0.0<br>6d  | 1.17±0.06<br>d | 0.59±0.03a | 1.80±0.0<br>9g  | 0.99±0.05<br>f | 1.21±0.06<br>d   | 0.80±0.04<br>e | 1.23±0.06<br>d   | 1.46±0.07<br>c   | 0.56±0.03<br>a |
| <b>Phloridzin</b>                    | 54.355 | 2.48±0.12a       | ND               | 11.3±0.6<br>e   | 4.40±0.22<br>c | 1.44±0.07d | 9.14±0.4<br>6f  | 1.53±0.08<br>d | ND               | 0.94±0.05<br>d | 8.57±0.43<br>f   | 3.27±0.16<br>b   | 1.60±0.08<br>d |
| <b>Ellagic acid</b>                  | 55.284 | <LOQ             | 39.8±2.0<br>a    | 21.3±1.1<br>c   | 20.9±1.0c      | ND         | 23.8±1.2<br>d   | ND             | 11.6±0.6b        | ND             | ND               | 13.0±0.65<br>b   | ND             |
| <b>3,4-di-O-caffeyolquinic acid</b>  | 56.781 | 33.5±1.7b        | 65.3±3.3<br>a    | 47.3±2.4<br>c,f | 32.3±1.6b      | 12.6±0.6e  | 58.4±2.9<br>a,g | 12.1±0.6e      | 106±5d           | 16.2±0.8e      | 16.4±0.8e        | 52.4±2.62<br>c,g | 42.3±2.1f      |
| <b>Cinnamic acid</b>                 | 58.47  | 4.10±0.21b       | 25.5±1.3<br>a    | 19.8±1.0<br>c   | 20.4±1.0c      | 1.46±0.07e | 9.89±0.4<br>9f  | 0.66±0.03<br>e | 3.57±0.18<br>b,d | 1.66±0.08<br>e | 2.31±0.12<br>d,e | <LOQ             | 4.60±0.23<br>b |
| <b>Kaempferol-3-O-glucoside</b>      | 59.466 | 2.73±0.14b       | 15.8±0.8<br>a    | 13.7±0.7<br>c   | <LOD           | ND         | 4.67±0.2<br>3d  | ND             | ND               | ND             | 8.64±0.43<br>e   | <LOD             | ND             |
| <b>Kaempferol-3-O-rutinoside</b>     | 60.277 | <LOD             | 9.72±0.4<br>9a   | 9.20±0.4<br>6a  | <LOQ           | ND         | 5.71±0.2<br>9c  | ND             | ND               | ND             | 7.47±0.37<br>d   | 1.40±0.07<br>b   | ND             |
| <b>Isorhamnetin-3-O-rutinoside</b>   | 61.568 | 4.75±0.24b       | 3.10±0.1<br>6a,c | 3.28±0.1<br>6c  | 2.84±0.14<br>a | ND         | 2.86±0.1<br>4a  | ND             | ND               | ND             | ND               | ND               | ND             |
| <b>Σ Phenolic compounds</b>          |        | 733              | 3440             | 3244            | 3196           | 3332       | 4600            | 2478           | 4219             | 2450           | 2241             | 3983             | 2494           |

LOD, limit of detection. LOQ: limit of quantification; ND, not detected. Within line different letters indicate significant differences ( $p < 0.05$ ) between samples.