

Article

Eco-Friendly Voltammetric Techniques for Assessing Antioxidant Properties in Dietary Supplements

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Abstract

Dietary supplements often promote their antioxidant content as an indicator of quality on the packaging. This study evaluated the redox potential and total antioxidant capacity of various dietary supplements, using different analytical methods to obtain the complexity of antioxidant measurements. A green approach for detecting total antioxidant capacity in dietary products utilized modern electrochemical techniques, including differential pulse voltammetry (DPV) and cyclic voltammetry (CV). These rapid “green” methods measure the redox potential of samples, providing information about the electron-donating ability of antioxidants without the use of harmful chemicals or sample treatments, with minimal environmental impact. ABTS and FRAP measurements were expressed as vitamin C equivalents to allow comparison with CV measurements and actual vitamin C content. This approach enabled indirect comparison of activities obtained using different standard substances through conversion to standard equivalents. The results revealed that the claims made on product labels and packaging often overestimated the antioxidant content and did not match the measured total antioxidant capacities obtained in the current study. Measured vitamin C levels in 10 samples fell within the declared ranges (0–950 mg), but six products contained 4.85% to 49.18% less, and two had significantly higher levels (4.20% and 32.22%) than their declared ($p < 0.05$). Total antioxidant capacity varied from the labeled values. Similar trends were observed across methods, except for DPPH. FRAP values were correlated with ABTS and CV ($r = 0.797$ and $r = 0.757$, respectively). The DPV method provided a more detailed assessment of the redox activity of selected products based on distinct oxidation peaks. The study highlights the importance of mandatory testing and quantification of antioxidants, as well as the need for regulation of antioxidant properties through normative standards.

Keywords: dietary supplement; redox potential; voltammetry; antioxidants

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1. Introduction

Antioxidants are molecules that play a crucial role in reducing oxidative damage to cells, protecting them from the harmful effects of free radicals. This can be achieved through direct reactions, inhibition of enzyme generation, or by enhancing the activity of intracellular antioxidant enzymes [1,2].

Oxidation and reduction are fundamental processes in biological systems. Free radical oxidants, such as reactive oxygen species, are produced inside cells through various

metabolic processes. The body is equipped with an antioxidant defense system that guards against oxidative damage caused by these reactive oxidants and plays a major role in protecting cells from oxidative stress and damage. Oxygen-derived radicals are generated constantly as a consequence of normal aerobic life. The imbalance between their production and the ability to neutralize them leads to oxidative stress of the organism and activates antioxidant defense mechanisms.

Nowadays, numerous dietary products are marketed as supplements to the normal diet, supposedly to help fulfill basic nutritional needs. Dietary supplements (DS) are concentrated sources of individual or a combination of physiologically and nutritionally active ingredients [3–5]. The antioxidants are widely accepted as a key factor in determining the quality of dietary supplements, as they enhance the biological value of the diet and have positive effects on the body [6–10]. The most commonly used antioxidants in these products are two types of non-enzymatic antioxidants, lipophilic (tocopherols, retinol, carotenoids) and hydrophilic (ascorbate–vitamin C, organically bound selenium, polyphenolics, low molecular weight thiols, plant extracts, animal-derived substances, such as bee products or bovine colostrum, enzymatic cofactors, coenzyme Q10). Their biological availability and effectiveness depend on concentration, chemical form, and interactions between different elements. The sum of antioxidant activities related to these molecules was earlier defined as total antioxidant capacity [2,11,12].

Despite the growing market for these products, there is a lack of standardized testing protocols, which leads to inconsistencies in labeling and confusion among consumers. Although some supplements claim to possess high antioxidant capacities, these claims often do not align with actual measurements [13–17]. The current protocols for measuring antioxidant capacity are complex, time-consuming, and lack standardization, leading to discrepancies in reported results. Therefore, a more reliable and standardized method is needed to ensure that label values accurately reflect the true antioxidant potential of the products [18,19]. The most common methods are spectrophotometric assays, including: 2,2-diphenyl-picrylhydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay, and 2,2-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay. These methods have limitations in applicability, selectivity, and sensitivity, making direct comparison difficult and often relying on reagents that can be hazardous [20,21]. A new approach for detecting total antioxidant capacity in dietary products utilizes modern electrochemical techniques, including differential pulse voltammetry (DPV) and cyclic voltammetry (CV). These methods measure the redox potential of samples, providing information about the electron-donating ability of antioxidants without the use of harmful chemicals and sample treatment. They are considered “green techniques” because they have minimal environmental impact, as they do not use organic solvents, allow for rapid analysis, and are reliable and cost-effective [22,23]. These methods have found wide application in the rapid, reliable, and “green” analysis of the redox and antioxidant potential of food products. In addition to the fact that various methods can be developed for the selective and highly sensitive analysis of individual components based on chemically modified electrodes, the application of electrochemical methods for monitoring antioxidant capacity without complex sample preparation and with minimal consumption of additional chemicals has been demonstrated. This approach, using a standard glassy carbon electrode, enables a very high integration of these methods into modern trends in analytical and environmental chemistry [24–27]. Compared to amperometry, which is a dominantly quantitative method, cyclic voltammetry can be applied for quantitative and qualitative analysis of redox processes, providing information on oxidation and reduction potentials and the reversibility of reactions. Oxidation and reduction peaks in the voltammogram are used for the detection of active components, electron transfer

mechanisms, and diffusion or kinetic properties. Chronoamperometry, on the other hand, measures current as a function of time after a potential step and is mainly used to study kinetics. Compared to previous methods, differential pulse voltammetry shows higher sensitivity and lower detection limits compared to CV and amperometry, as it applies a pulsed signal that minimizes background current. It effectively separates closely spaced oxidation peaks, allowing the detection of multiple antioxidant components in complex matrices and highly accurate quantitative analysis. It can detect redox potential within a mixture, which is especially important for complex samples such as dietary supplements or herbal extracts [28,29].

Analysis of such samples is a necessity in the current supplement market, as their consumption has increased dramatically. Most manufacturers declare the content of antioxidant components without specifying the method of determination. The existence of a standardized method as well as rapid and commercially available tests for this type of analysis is of great importance for consumers. Based on previous studies demonstrating that the electrochemical methods can be successfully applied in complex matrices such as wine, honey, milk, or infant formula [24–28], the main hypothesis was to try to apply and validate green electrochemical methods as potential standard approaches for detecting antioxidant potential of dietary supplements, a field highly relevant to health care, yet lacking standardized analytical criteria. This study aims to compare different methods for determining the total antioxidant capacity of dietary supplements, in order to obtain reliable values that can be used to assess product value based on antioxidant content. By evaluating both traditional spectrophotometric assays and modern electrochemical techniques, this study aims to identify the most accurate methods for determining total antioxidant capacity and verify the accuracy of claims related to antioxidant capacity. This will support more accurate labeling practices and help prevent consumer misconceptions about the effectiveness of dietary supplements.

2. Materials and Methods

2.1. Sample Preparation

Eighteen dietary supplements with label claims that they contained antioxidants were selected from various manufacturers on the market, including 10 domestic (D) and eight imported (I) products. Selection focused on widely available, commonly used, and reputable supplements, with diverse ingredients, a clear and complete list of ingredients, precise dosages, and an emphasis on quality and safety. The selected dietary supplements were available on the market in various pharmaceutical forms: capsules (39%), powders (28%), tablets/pastilles (28%), and solutions (5%). Products were based on different natural plant products like polyphenols, carotenoids, astaxanthin, bioflavonoids, lutein, resveratrol, and lycopene, mushroom extracts, or animal-derived products, propolis, royal jelly, with added vitamins (C, D, E, and A), and minerals, Zn, and Se. The list of biologically active ingredients declared on the labels of the selected products, the test dose (this is the recommended daily use dose), and the mass of the test dose are shown in Table 1. Dietary supplements were dissolved at the recommended dose in 250 mL (sometimes specified as a “standard glass of drinking water”) of deaerated, demineralized water, to avoid different drinking water compositions (Figure 1). L-ascorbic acid (vitamin C, 500 mg) was used as a reference substance. All chemicals and reagents used in this study were of analytical reagent grade.

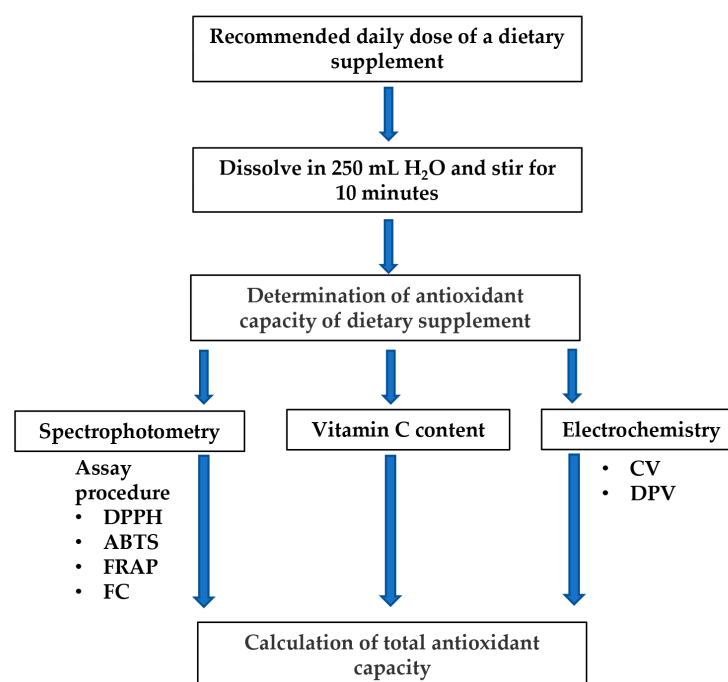


Figure 1. Scheme of the experimental protocol.

Table 1. Biologically active ingredients, pharmaceutical form, and dose of dietary supplements.

Dietary Supplement	Biologically Active Ingredients	Quantity/Test Dose	Mass of Test Dose
1D *	Polyphenols from <i>Aronia melanocarpa</i>	1 Solution	400 mg
2D	Dry ethanol extract of propolis; Vitamin C, E, B1, D3, β -Carotene; Minerals: Zn, Se	2 Capsules	1170 mg
3D	Astaxanthin; Vitamin C; Minerals: Se, Zn	1 Dry granules	3000 mg
4D	Propolis extract; Vitamin C	1 Tablet	830 mg
5D	Dry ethanol extract of propolis; Vitamin C, E, β -Carotene	1 Tablet	830 mg
6D	Mineral: Zn; Vitamin: C	1 Capsule	130 mg
7D	Mineral: Se; Vitamin C, E, A	1 Capsule	290 mg
8D	Ground and activated seed of <i>Brassica oleracea</i> var. acephala; Minerals: Zn, Cu; Vitamin C; Inulin	1 Capsule	530 mg
9D	Royal Jelly; Vitamin C	1 Dry granules	11,000mg
10D	Royal Jelly; Vitamin C	1 Dry granules	12,000 mg
1I	Bioflavonoids, Lutein, Lycopene; Vitamin A, D, E, K, C, B1, B2, B6, Niacin, Biotin, Folic acid; Minerals: K, Mg, Fe, Zn, Cu, Mn, Se, Cr, Mo, I	3 Tablets	3600 mg
2I	Bioflavonoid, Lutein, Lycopene; Vitamin A, D, E, K, C, B1, B2, B6, B ₁₂ , Niacin, Biotin, Folic acid; Minerals: Na, Fe, Zn, Cu, Mn, Se, Cr, Mo, I	1 Dry granules	15,000 mg

Table 1. Cont.

Dietary Supplement	Biologically Active Ingredients	Quantity/Test Dose	Mass of Test Dose
3I	Vitamin D, Calcium L-ascorbate; Mineral: Zn	1 Capsule	600 mg
4I	Mushroom extract, resveratrol, lycopene, Royal jelly	1 Capsule	600 mg
5I	Vitamin A, C, E; Minerals: Se (Selenium yeast)	1 Capsule	480 mg
6I	Colostrum bovine; Vitamin D3	1 Dry granules	950 mg
7I	Vitamin A, C, E, D, B1, B2, B3, B6, B9, B12, Calcium-pantothenate	1 Tablet (dragee)	1100 mg
8I	Vitamin C, D; Mineral: Zn	1 Chewing tablet	2220 mg
Reference substance	L-ascorbic acid	Powder	500 mg

* D indicates domestic products, I indicates imported products.

2.2. Cyclic Voltammetry and Differential Pulse Voltammetry

Green electrochemical methods were employed for detecting the redox capacity in dietary supplements, utilizing cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The results of the electrochemical methods were expressed as equivalent of vitamin C (CE). Voltammograms for cyclic and differential pulse voltammetry were recorded on the CH1760B instrument (CHInstruments, Austin, TX, USA). All measurements were performed in a three-electrode cell/quartz cuvette, where a glassy carbon (GC) electrode was used as the working electrode (Model CHI 104), and an additional large surface platinum electrode (Model CHI 221) and Ag/AgCl electrode (Model CHI 111) were used as the auxiliary and reference electrodes. The electrochemical cells had a volume of 5 mL. To ensure identical conditions throughout the experiment, each sample was diluted with 0.1 M KCl solution in a 1:1 ratio. Measurements were performed in an anaerobic atmosphere with N₂ introduced into the cell. The working electrode was polished with alumina powder (1 and 0.5 µm, Buehler, IL, USA), rinsed with ethanol and deionized water, prior to each measurement. The voltammograms were recorded in the range of 0 to 1000 mV, with a scan rate 100 mV/s for CV, and 1 mV/s for DPV method. All measurements were performed at room temperature (20 °C). To produce a calibration curve for vitamin C, voltammetric cells contained 5000 µL of the supporting electrolyte (0.1M aqueous KCl solution) plus appropriate volumes of a 0.01M vitamin C solution. Measurements were performed using an Ag/AgCl reference electrode, a GC working electrode, and a platinum wire auxiliary electrode. CV and DVP voltammograms of vitamin C were taken from two peaks, approximately at 350 mV and 650 mV. Calibration curves based on peak area (Ah) were from CV and DPV measurements (at 350 mV) and provided in Figure 2A,B, respectively. In the inset Figure 2A,B, corresponding calibration equations for DPV and CV methods were provided: $I(A) = 0.0015 \times c(M) + 5.4234 \times 10^{-7}$ ($R^2 = 0.9725$); $I(A) = 3.57 \times 10^{-7} + 0.013 \times c(M)$ ($R^2 = 0.9813$), respectively. All results were recorded in triplicate, and the relative standard deviation was below 4.7%, indicating a significantly high reproducibility of the method. The detection limit (calculated as $3\sigma/\text{slope}$) and quantification limits (calculated as $10\sigma/\text{slope}$) for both methods were as follows: $\text{LOD}_{\text{CV}} = 0.9 \mu\text{M}$ and $\text{LOQ}_{\text{DPV}} = 2.9 \mu\text{M}$; $\text{LOD}_{\text{DP}} = 0.05 \mu\text{M}$ and $\text{LOQ}_{\text{DP}} = 0.19 \mu\text{M}$.

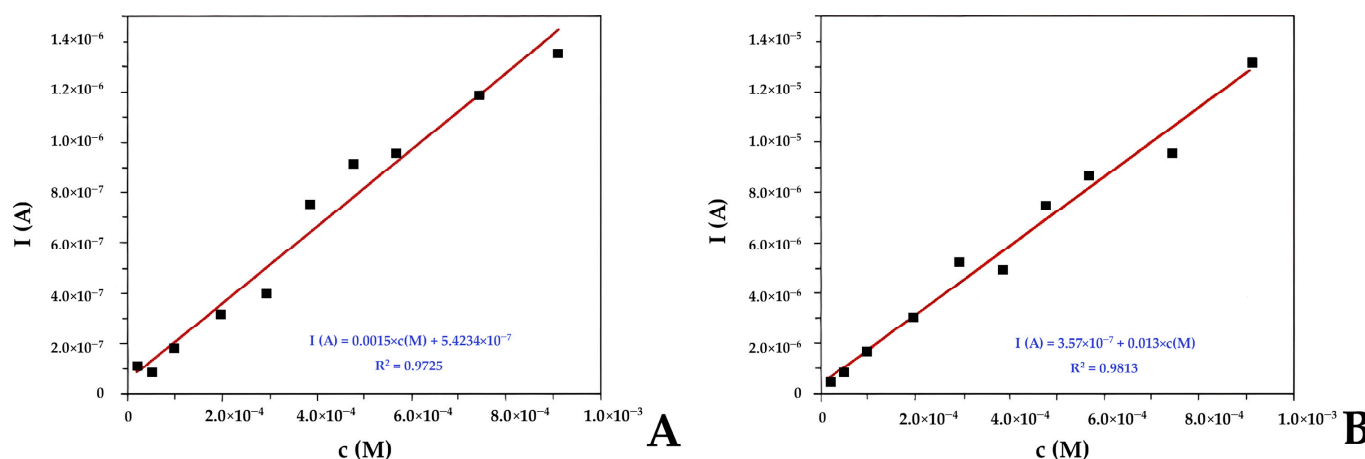


Figure 2. Calibration curves for different concentrations of vitamin C obtained using (A) differential pulse voltammetry, and (B) cyclic voltammetry.

2.3. Methods for the Detection of Antioxidant Properties

The antioxidant properties of dietary supplements were determined by spectrophotometric methods: DPPH, Folin–Ciocalteu’s assay for phenolic substances and flavonoids, the FRAP assay for ferric reducing antioxidant power, and ABTS assay for the ability of antioxidants to scavenge the ABTS radical cation. Vitamin C content of selected products was detected using a Reflectometer RQflex® 20 (Supelco) system (Merck, Darmstadt, Germany), according to the manufacturer’s protocol, and results were expressed as mg of vitamin C per 250 mL of solution. pH values were detected using a pH meter (WTW, Weilheim, Germany).

The radical scavenging activity of supplements was detected at 517 nm (UV/VIS Spectrometer UV1280, Shimadzu), after incubation with DPPH solution (0.1 mmol/L) for 30 min at room temperature in the dark and expressed as percent of scavenging activity per 250 mL of solution [30].

The phenolic content of product samples was obtained after the addition of Folin–Ciocalteu reagent, left for 6 min before the addition of Na_2CO_3 . After incubation for 2 h at room temperature, the absorbance was measured at 765 nm. The total phenolic content was expressed as gallic acid equivalent (GAE) per 250 mL of solution [31].

The ABTS antioxidant capacity of the products was determined by a colorimetric assay using the reduction in $\text{ABTS}^{\bullet+}$ radical cation, generated overnight with potassium persulfate. The results were detected spectrophotometrically at 734 nm and expressed as mg of Vitamin C equivalent (CE) per 250 mL of solution [32].

The FRAP was determined under acidic conditions following the reduction in the ferric 2,4,6-tripyridyl-s-triazine complex. Antioxidant activity was measured spectrophotometrically at 593 nm and expressed as mg of Vitamin C equivalent (CE) per 250 mL of solution [33].

2.4. Statistical Analysis

All chemical analyses were conducted independently in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD). Statistical analyses were carried out using SPSS ver. 25 software (IBM, Armonk, New York, NY, USA). A repeated measures ANOVA was applied to assess differences among the analytical methods for determining antioxidant capacity. Wilk’s test was used to test the overall model significance. When significant differences were observed ($p < 0.05$), pairwise comparisons were performed to identify specific differences between methods.

3. Results

A total of 18 dietary supplements labeled as containing antioxidants, from various manufacturers available on the Serbian market, were analyzed. The composition of the products differed, with 12 containing natural extracts from plants, fungi, propolis, royal jelly, and bovine colostrum. The remaining six products were formulated based on vitamins and minerals. The mass of daily dosage also differed, ranging from 130 mg to 15 g for solid forms, and up to 30 mL for liquid solutions (Table 1). These findings highlight the diversity of antioxidant sources and dosage forms in widely consumed supplements and provide insight into their potential nutritional and health contributions.

The measured vitamin C content for 10 samples fell within the declared ranges (Figure 3). However, samples 8D, 9D, 10D, 1I, 3I, 7I, and 8I exhibited lower vitamin C levels (24.22%, 4.85%, 13.90%, 39.34%, 15.91%, 31.11%, and 49.18%, respectively), while samples 2D (4.20%) and 6D (32.21%) exhibited statistically significant higher levels than their declared vitamin C contents ($p < 0.05$). Samples 1D, 4I, and 6I did not have any declared vitamin C contents. In sample 4I, based on mushroom extract, royal jelly, resveratrol, and lycopene (4I), a low level of vitamin C was detected (16 mg), while vitamin C was not detected in colostrum-based sample. Both samples had pH values (7.07 ± 0.01 and 6.86 ± 0.01 , respectively). Product based on extract of *Aronia melanocarpa* (1D) contained 86 ± 6 mg vitamin C, although it did not have a declared vitamin C content. In addition to this sample, two other samples showed high measured values of vitamin C compared to their declared contents. Sample 2D exhibited a 4.20% higher vitamin C level than declared, while the vitamin C in sample 6D was 32.22% higher than the label declaration (Appendix A).

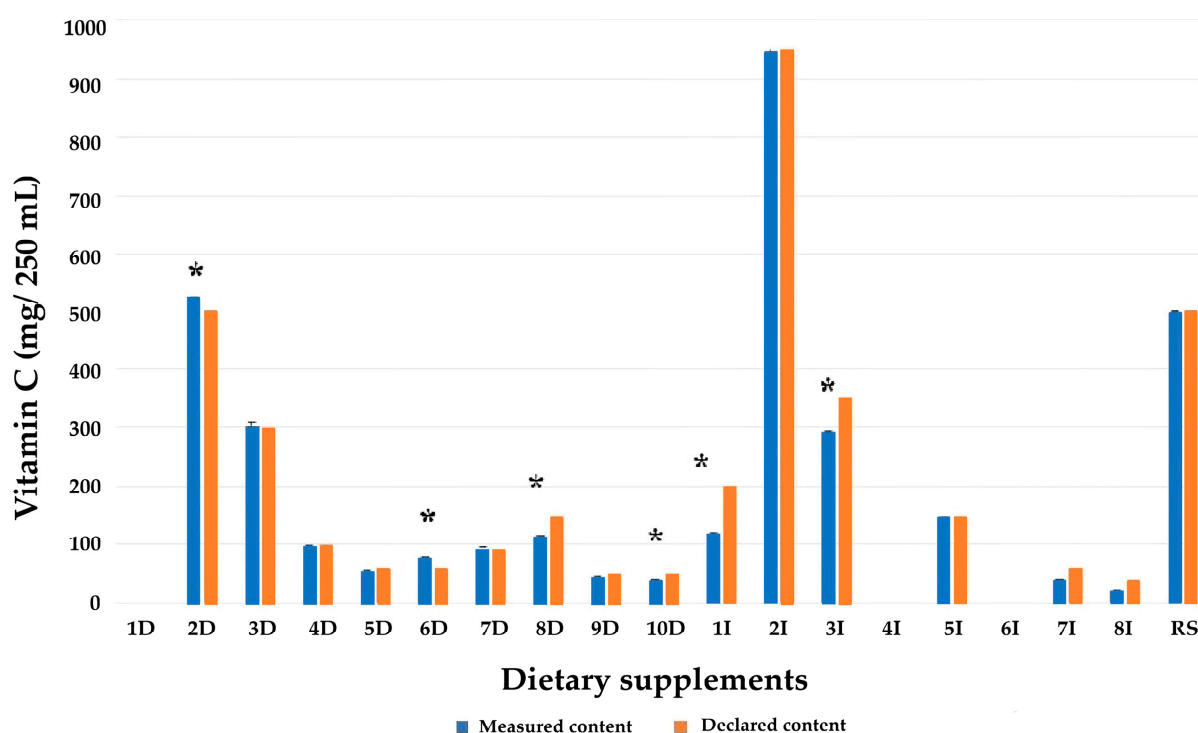


Figure 3. Comparison of measured and declared vitamin C content in the examined dietary supplements. D indicates domestic products, I indicates imported products. * indicates a statistically significant difference between measured and declared content ($p < 0.05$).

The antioxidant and redox potentials of selected products were compared by four spectrophotometric methods and one electrochemical method, specifically cyclic voltammetry (Figure 4). When the total antioxidant capacity was assessed using the

FRAP assay, the products 2I, 3I, and 2D displayed very high activity, while 1D and 3D showed notable levels. In contrast, 4D-7D, 1I, and 4I exhibited lower total antioxidant capacity. The Folin test indicated there was polyphenol activity in all products, expressed as GAE. The highest activity was observed in 2I, followed by 2D, 6D, 1D, 5D, and 3I, which also exhibited high activity. The total antioxidant capacities obtained by CV agreed well with those from the ABTS assay. With the CV method, the highest reductant properties were detected in 2D and 2I, indicating strong antioxidant capacity compared to the other products with lower antioxidant concentrations.

The highest redox activities were detected in a propolis-based product containing vitamin C, E, D, B, and β -carotene, and in a plant flavonoid-based product containing lutein, lycopene, and B vitamins (2D and 2I, respectively). In contrast, the animal-derived colostrum dietary product, 6I, showed very low total antioxidant capacity, as measured by both spectrophotometric and electrochemical methods. The DPPH method produced exceptionally high scavenging activity for all products, ranging from 89% to 97%, while the activity for 500 mg of vitamin C per 250 mL of water was 98%. The colostrum product, 6I, and the product with Zn and vitamin C (6D) exhibited extremely low total antioxidant capacity (9% and 13%, respectively), making the DPPH method the least reliable of the methods for determining the total antioxidant capacity of the products.

However, except for DPPH method, a similar trend was observed for total antioxidant capacity across the analytical methods, for both highly- and less-active dietary supplements (Figure 4). The ABTS assay exhibited the highest total antioxidant capacities compared to other methods and vitamin C contents (1D, 2D, 5D, 7D, 2I, 3I, 4I), whereas the lowest total antioxidant capacities and vitamin C contents were measured in products 8D and 6I. In some products, the vitamin C levels corresponded to the total antioxidant capacity measured using the CV method (4D, 5D, 7D, 8D, 3I, 5I, and for the vitamin C standard), while the CV method resulted in considerably lower total antioxidant capacities for products that exhibited vitamin C levels below 60 mg (1D, 6D, 9D, 10D, 1I, 4I, 6I, 7I, and 8I). Figure 4F presents the distribution of total antioxidant capacity in the analyzed supplements, as determined by different antioxidant methods. Statistical analysis of method comparison revealed differences in variability, i.e., sensitivity, among the tested methods. An inspection of the graph shows that the FC method followed the trend observed in all other methods; however, due to differences in the measurement scales, it could not be included in the ANOVA. Furthermore, the DPPH method exhibited an almost linear response that did not align with the trends of the other methods, and therefore, this method was excluded from the ANOVA. Comparison of three methods, ABTS, FRAP, and CV, based on repeated measures ANOVA, Pairwise Comparisons showed that there was no statistically significant difference between FRAP and ABTS or FRAP and CV ($p > 0.05$). CV values were correlated with FRAP ($r = 0.757$), and ABTS values were correlated with FRAP ($r = 0.797$). Still, Wilk's lambda analysis showed the ABTS method statistically differed from the CV method ($p < 0.05$).

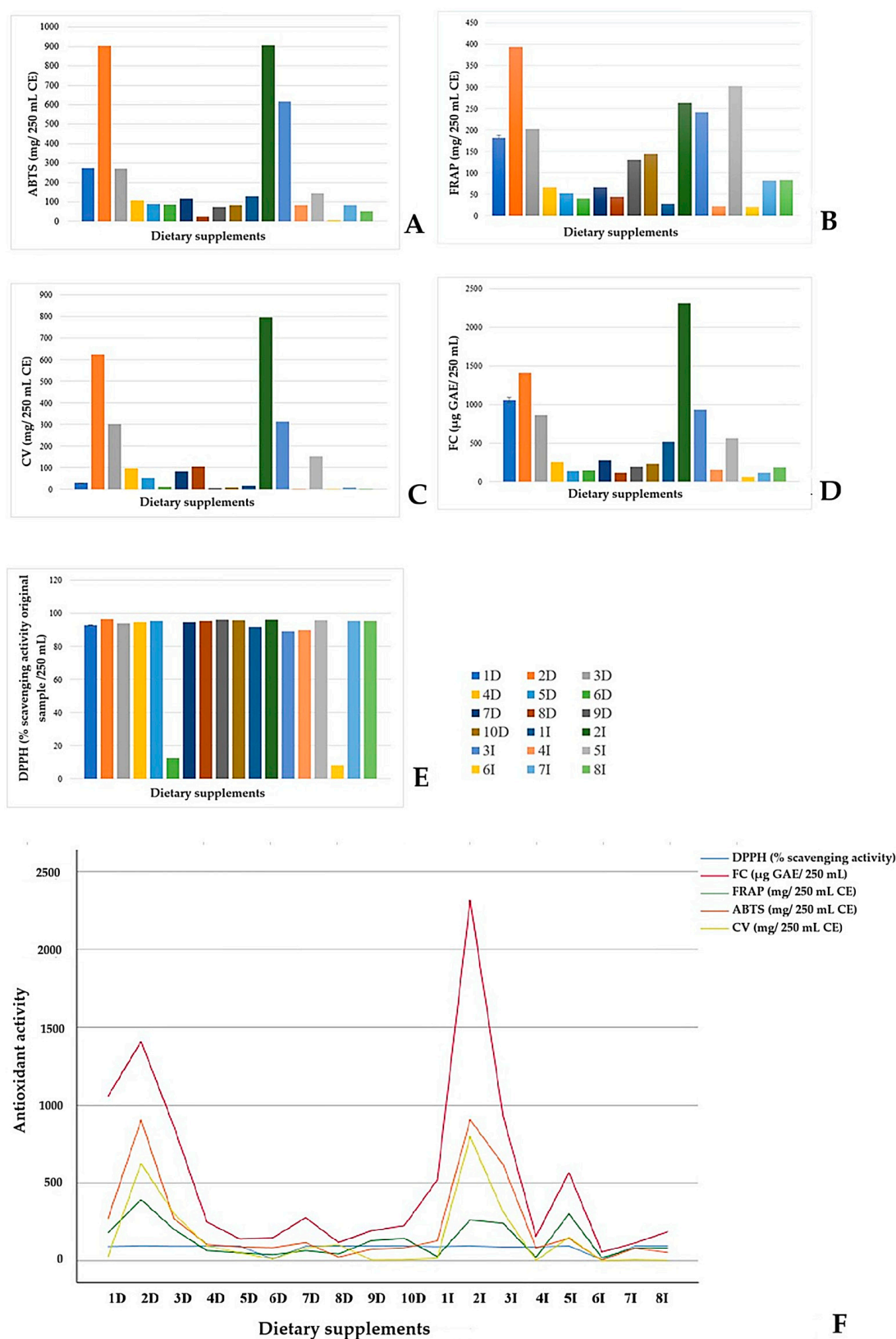


Figure 4. The total antioxidant capacity of selected products determined by four spectrophotometric methods and one electrochemical method: (A) ABTS radical scavenging activity, (B) Ferric Reducing Antioxidant Potential (FRAP), (C) cyclic voltammetry, (D) Folin–Ciocalteu assay, and (E) DPPH radical scavenging activity. Comparison of the distribution of total antioxidant capacity in dietary supplements determined by the selected methods (F). D indicates domestic products, I indicates imported products.

Figure 5 shows the summarized redox potential, expressed as total antioxidant capacity, of the tested dietary products when measured by the CV method. This method showed that products 2D and 2I had high redox potentials compared to the other tested products. When the CV method was used, only product 2D had a total antioxidant capacity higher than the declared vitamin C level. The total antioxidant capacity measured using the CV method did not show differences among samples 3D, 4D, 5D, 7D, 8D, 3I, 5I, and 6I with regard to their declared vitamin C content. Two samples, 2D and 2I, exhibited total antioxidant capacity values higher than the declared or measured vitamin C contents. In contrast, very low vitamin C levels were detected in declared and measured samples 1D, 6D, 9D, 10D, 1I, 4I, 6I, 7I, and 8I. However, this method has shown that the content of active compounds varies among most samples. According to the data on the samples, the dominant content of antioxidant compounds in the samples is different and is based on vitamin C, propolis, plant extracts and polyphenols. The electrochemical profile obtained from this study corresponds to that distribution and electrochemical behavior of the mentioned compounds [34–36]. This means that the dominant carriers of antioxidative activities are ascorbate and phenolic compounds, while other components are probably present to a lesser extent but contribute to the overall value. Similar considerations have been made in the works of other authors in the literature [34–37].

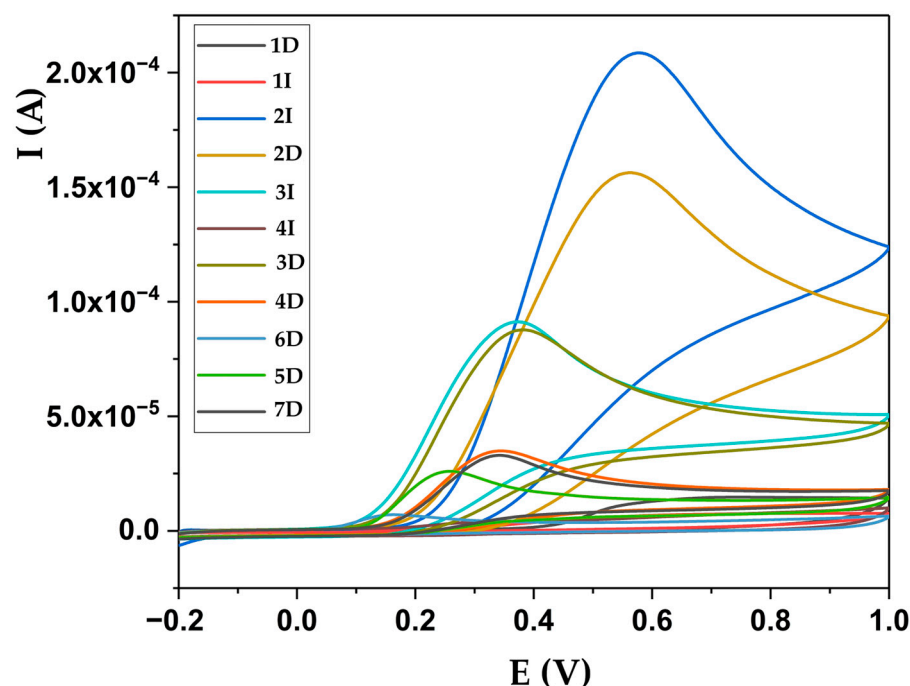


Figure 5. Redox potential of selected dietary products measured using cyclic voltammetry. D indicates domestic products, I indicates imported products.

Figure 6 illustrates two selected products, 2D and 2I, which exhibited the highest redox potential, along with a domestic product characterized by low redox potential, based on CV measurements (1D). Further analysis used a more sensitive DPV method enabling the identification of active components in the products, as demonstrated by product 2I, with two distinct oxidation peaks (E_{OX1} and E_{OX2}). The DPV analysis revealed that 2I contained various active components active at potential 0.385V and 0.425V, influencing the overall redox potential of the product. For the 2D sample, the existence of two distinct peaks (a broad peak representing the superposition of two peaks, (E_{OX1} and E_{OX2})) is also evident, which are the dominant carriers of the antioxidant activity of the sample, but which cannot be clearly separated due to the close value of the maximum potential.

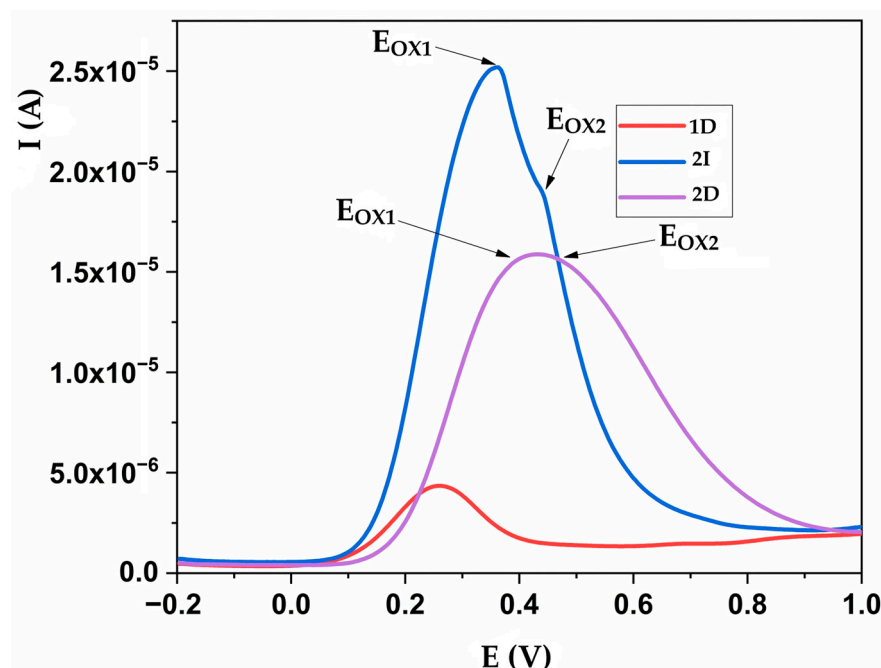


Figure 6. Redox potential of selected dietary products measured by differential pulse voltammetry. E_{OX1} and E_{OX2} represent distinct oxidation peaks of a broad peak. D indicates domestic products, I indicates imported products.

The CV method worked over a wide range of concentrations of substances that are responsible for the total antioxidant capacity of the dietary supplements, but, as previously known, it did not show satisfactory sensitivity when the products contained low levels of the substances. In addition, the CV method did not differentiate oxidation peaks and did not produce a “fingerprint” for each product. In contrast, the DPV method showed better sensitivity, as shown in Figure 6. This method produced a significant and measurable response for products where the CV method showed shortcomings. In addition, the appearance of the signal obtained with the DPV method was such that, in addition to quantification, this method produced some additional information about the product, i.e., on the existence of different components that contribute to the total antioxidant capacity, and the mutual concentration ratio.

The DPV method provided a more detailed assessment of the redox activity of the products, enabling the determination of redox potential based on distinct oxidation peaks. A limitation of the DPV method is that high concentrations of redox-active compounds cannot be measured directly without prior sample dilution, unlike the CV method. However, samples 1D, 6D, 9D, 10D, 1I, 4I, 6I, 7I, and 8I had declared vitamin contents below 60 mg/250 mL, which fell below the detection limit of the CV method and were, thus, considered out of range.

4. Discussion

The results indicate that the statements in the materials accompanying the products or in the inscriptions and the graphic representations on the packaging regarding the antioxidant content were, to put it mildly, more optimistic than the actual measurements obtained in this study. Thus, for some classes of compounds exhibiting redox properties, such as antioxidants, the reported values were lower by several fold in some of the analyzed dietary supplements than was claimed. The mass and active substance content differed among the different products. Although the study was limited by a small number of samples, it covered a variety of antioxidant sources and dosage forms in widely consumed supplements, including both locally produced and imported products available in Serbia.

These findings provide insight into their potential nutritional and health contributions. Therefore, any claim about the total antioxidant capacity of a product should be defined based on its specific activity and appropriately labeled with a standardized value.

The determination of total antioxidant capacity is primarily based on the reaction kinetics or equilibrium states of chemical assays, as assessed through spectrophotometric measurements. Antioxidant activity expressed as one standard equivalent can be converted to another using a conversion formula, allowing comparison between results based on different standards [38]. These measurements rely on detecting color changes or discoloration at defined wavelengths. Such assays have been widely utilized for antioxidant analysis and for evaluating the antioxidant capacity of complex biological and chemical samples. According to the results of the current studies, there is the same distribution of total antioxidant capacity between the studied methods. However, different methods have advantages and disadvantages for directly measuring antioxidant potential in undiluted, original dietary supplements. The DPPH method produced very high vitamin C levels for the products in which vitamin C was declared (results were 89–97% of declared levels). The FRAP method showed lower vitamin C levels compared to the ABTS and CV methods. The FC method provided insight into the activity of phenolic compounds in the tested dietary supplements, and the distribution of activity was consistent with the other methods. Previous research suggested that the DPPH method has several inconsistencies in the results it produces, making it unreliable for detecting antioxidant potential [27,39,40].

Electrochemical voltammetric methods are very sensitive for the detection of low concentrations of antioxidants, rapid measurement without sample preparation, calibration, and validation. Cloudy or complex samples with antioxidants can be analyzed to distinguish between different redox-active species, providing comprehensive insights into antioxidant mechanisms. Electrochemical methods are a valuable tool for both qualitative and quantitative antioxidant detection. CV measures current intensity, reflecting the redox activity of antioxidants. The DPV method requires prior sample dilution, making it unsuitable for determining the redox potential of highly active samples. Furthermore, the low pH of these products suggested the predominant presence of ascorbic acid, which would contribute to their high antioxidant potential. A limitation of the DPV method is that high concentrations of redox components cannot be measured directly without sample dilution, unlike the CV method. Disadvantages of CV and DPV are complex calibration and results interpretation, making them less practical for routine analyses.

ABTS and FRAP measurements were expressed as vitamin C equivalents to allow comparison with CV measurements and actual vitamin C content. The converted total antioxidant capacity of different products was consistent with the capacity measured by each individual method. This approach enabled indirect comparison of activities obtained using different standard substances through conversion to standard equivalents. The DPV method was not included in the comparison, and its results were not reported, as it is highly sensitive to low concentrations, while concentrations higher than 60 mg/250 mL require prior dilution, making standardization difficult. The CV method is suitable for antioxidant concentrations exceeding 60 mg/250 mL (daily dose), whereas the DPV method is more appropriate for detecting lower concentrations without prior treatment. In addition, the appearance of the signal obtained with the DPV method was such that, in addition to quantification, this method produced some additional information about the product, i.e., on the existence of different components that contribute to the total antioxidant capacity, and the mutual concentration ratio, which would allow individual samples to be grouped. Therefore, this method may be useful for recognizing the type of dietary supplement or the compounds that have significant antioxidant activity in the supplement, such as dietary

products with bovine colostrum compared to high active human colostrum and breast milk [27].

While special medical conditions, deficiencies, or increased need require supplementation, whole foods are superior for overall health due to their complex nutrient composition, bioavailability, and natural synergy. The comparative efficacy of dietary products and fresh groceries with regard to antioxidant potential remains an open question, although current data suggest that fresh vegetables and fruits could offer superior antioxidant potential. Vitamin C, a common ingredient of dietary supplements with antioxidant properties and with enormous potential to prevent the development of oxidative reactions of macromolecules, is by definition a reductant. The emphasis on the significance of vitamin C content is somewhat relevant [6,9,41]. However, this compound in supplements does not necessarily contribute more antioxidant value than natural sources like fresh red peppers. This vegetable contains not only the antiscorbutic vitamin, but it also possesses the reducing agents that neutralize free radicals and oxidative stress.

Dietary supplements' antioxidant effectiveness, in comparison with fresh whole foods, such as vegetables, is often overstated and should not be understood by consumers as replacements for a whole food diet that includes suitable quantities of vegetables and fruits. The discrepancy between declared and measured antioxidant capacities in dietary supplements may lead to significant underexposure or overexposure to certain redox-active compounds. Inaccurate labeling can mislead consumers regarding the actual intake of antioxidants, potentially resulting in insufficient efficacy or, conversely, unintended excessive intake. Given that some products exceeded or fell below the recommended daily allowance, such deviations may pose a risk to vulnerable populations, including pregnant women, elderly individuals, and those with chronic conditions. These findings underscore the need for mandatory quantification and accurate labeling of antioxidant content to minimize consumer health risk and ensure product efficacy.

5. Conclusions

This study compared standard spectrophotometric methods with eco-friendly electrochemical methods for determining the concentration of antioxidants in dietary supplements and evaluated their agreement with the manufacturers' declared values. Given the current absence of a standardized method for the detection of total antioxidant capacity, the use of the electrochemical "green" cyclic voltammetry and differential pulse voltammetry methods showed potential use as faster, simpler, and more sustainable analytical approaches that require minimal use of additional chemicals and enable direct measurements. The correlation between differential pulse voltammetry and cyclic voltammetry should be considered in future studies, and further work is needed to develop the most reliable approach. Also, this study showed the shortcomings in the proper definition of the antioxidant content of various supplements, as well as the existence of a clearly defined method for determining their content in these samples. Each of the methods showed significant deviations from the declared values but excellent mutual agreement, which leads to the conclusion that this field is still very undefined. In some cases, labeling is not based on the concentration of bioactive compounds or the true characteristics of the product, which may mislead consumers. Therefore, this study highlights the need for normative regulation of antioxidant properties through the introduction of mandatory testing and quantification of total antioxidant capacity.

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Abbreviations

The following abbreviations are used in this manuscript:

ABTS	2,2-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid
CE	Vitamin C equivalent
CV	Cyclic voltammetry
D	Domestic product
DPPH	2,2-diphenyl-picrylhydrazyl
DPV	Differential pulse voltammetry
DS	Dietary supplements
FC	Folin-Ciocalteu
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
GC	Glassy carbon
I	Imported product

Appendix A

Table A1. Comparison of results from antioxidant assay methods, vitamin C equivalents, and pH values.

Dietary Supplement	DPPH (% Scavenging Activity Original Sample/ 250 mL)	FC (µg GAE/ 250 mL)	FRAP (mg/250 mL CE)	ABTS (mg/250 mL CE)	CV (mg/250 mL CE)	Vitamin C (mg/250 mL Daily Dose)	Vitamin C (Declared, mg)	pH
1D	92.90 ± 0.10	1058.67 ± 35.92	181.33 ± 6.11	273.33 ± 5.86	29.60 ± 0.54	86.00 ± 6.00	/	3.35 ± 0.01
2D	96.50 ± 0.50	1409.67 ± 58.51	393.67 ± 7.23	904.00 ± 41.07	625.50 ± 25.43	521.33 ± 3.51	500	3.61 ± 0.01
3D	93.97 ± 0.15	861.33 ± 33.56	202.33 ± 6.81	270.17 ± 12.75	303.67 ± 21.78	304.67 ± 4.51	300	4.29 ± 0.02
4D	94.93 ± 0.06	250.33 ± 41.96	66.33 ± 1.53	106.83 ± 13.04	97.00 ± 1.00	97.01 ± 2.02	100	3.86 ± 0.01
5D	95.67 ± 0.29	140.67 ± 25.66	52.33 ± 1.53	90.17 ± 2.25	52.08 ± 0.88	54.67 ± 2.51	60	3.31 ± 0.01
6D	12.67 ± 0.15	147.67 ± 13.05	40.33 ± 0.58	85.33 ± 7.51	11.67 ± 0.58	79.33 ± 1.15	60	4.62 ± 0.02
7D	94.67 ± 0.42	278.33 ± 16.20	66.33 ± 3.21	117.33 ± 7.51	83.25 ± 5.06	92.67 ± 3.78	90	3.41 ± 0.02
8D	95.57 ± 0.67	118.33 ± 8.39	44.67 ± 0.58	24.833 ± 2.02	104.83 ± 1.26	113.67 ± 1.53	150	4.17 ± 0.02
9D	96.30 ± 0.26	193.67 ± 3.21	130.33 ± 2.08	74.83 ± 7.29	5.93 ± 0.38	45.67 ± 0.58	48	3.05 ± 0.02
10D	96.03 ± 0.55	227.33 ± 8.74	144.67 ± 3.79	84.33 ± 5.13	6.97 ± 0.42	41.33 ± 1.15	48	3.15 ± 0.01
1I	91.67 ± 0.58	519.33 ± 17.50	28.33 ± 0.58	129.33 ± 11.02	16.00 ± 2.14	121.33 ± 1.15	200	5.86 ± 0.02
2I	96.33 ± 0.58	2316.33 ± 48.85	263.33 ± 3.79	906.33 ± 35.13	797.67 ± 32.47	947.00 ± 3.01	950	3.35 ± 0.01
3I	89.07 ± 0.06	931.33 ± 38.85	241.33 ± 4.93	618.33 ± 29.28	314.42 ± 19.99	294.33 ± 0.58	350	6.20 ± 0.0
4I	89.93 ± 0.21	157.33 ± 18.93	22.67 ± 0.58	83.33 ± 5.20	3.11 ± 0.16	15.67 ± 0.58	/	7.07 ± 0.01
5I	95.77 ± 0.32	566.33 ± 16.50	302.33 ± 3.21	144.33 ± 9.02	151.50 ± 6.26	148.33 ± 1.53	150	4.97 ± 0.01
6I	8.16 ± 0.97	57.67 ± 15.04	20.67 ± 2.51	3.42 ± 0.72	0.27 ± 0.03	0	/	6.86 ± 0.01
7I	95.57 ± 0.81	114.67 ± 7.02	82.33 ± 3.06	81.67 ± 12.58	7.01 ± 0.01	41.33 ± 1.15	60	3.98 ± 0.01
8I	95.57 ± 0.60	186.67 ± 14.50	83.67 ± 3.21	53.67 ± 6.03	3.01 ± 0.01	20.33 ± 0.58	40	3.71 ± 0.01

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