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Determination of the Relative Contribution of Quercetin and Its Glucosides to the Antioxidant Capacity of Onion by Cyclic Voltammetry and Spectrophotometric Methods

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This paper describes the use of cyclic voltammetry (CV), spectrophotometric methods [Trolox equivalent antioxidant capacity (TEAC), peroxyl radical trapping capacity (PRTC), DPPH radical scavenging activity (RSA), and Folin−Ciocalteu reagent (FCR) reducing capacity], and photochemiluminescence (PCL) for the measurement of the antioxidant capacity of onion var. Sochaczewska and var. Szalotka. The antioxidant and reducing activity of the dominant onion flavonoids quercetin (Q), quercetin-3-*O*-β-glucoside (Q3G), quercetin-4′-*O*-β-glucoside (Q4′G), and quercetin-3,4′-di-*O*-β-glucoside (Q3,4′G) were determined by spectrophotometric (TEAC and PRTC) and CV methods, respectively. The contribution of quercetin and its glucosides to the antioxidant capacity of onion was calculated in consequence of the qualitative and quantitative analysis of onion flavonoids by high-performance liquid chromatography−ultraviolet−mass spectrometry. The dominant forms of quercetin in the onion var. Sochaczewska and Szalotka included Q4′G (61 and 54%), Q3,4′G (37 and 44%), Q3G (1.4 and 1.1%), and free quercetin (1.1 and 0.7%), respectively. The CV experiment showed the highest reducing activity of Q while Q3G, Q4′G, and Q3,4′G exhibited about 68, 51, and 30% of the reducing power noted for Q. The order of the reducing activity of onion flavonoids was confirmed by their free radical scavenging activity and evaluated by TEAC and PRTC assays as follows: Q > Q3G > Q4′G > Q3,4′G. The Q4′G and Q3,4′G showed poor antioxidant activity under both applied spectrophotometric assays but still exhibited reducing activity based on CV experiments. The reducing capacity of onions determined by CV method was twice higher than the antioxidant capacity formed by water-soluble compounds (ACW) evaluated by PCL, and it was about 50% higher than PRTC and DPPH RSA results and the converted FCR reducing capacity. In contrast, the reducing capacity of onions determined by the CV method was 3-fold and about four times lower when compared to the antioxidant capacity evaluated by the TEAC method and that formed by lipid-soluble compounds (ACL) provided by PCL, respectively. The highest antioxidant capacity of onion was found under cumulative consideration of PCL (ACW + ACL) and TEAC assays. The relative contribution of Q and its glucosides to the antioxidant capacity of onions showed a low contribution of Q, Q3G, and Q3,4′G derived from CV, TEAC, and PRTC assays while the highest contribution to the antioxidant capacity of onions was provided by Q4′G.

KEYWORDS: Quercetin; quercetin glucosides; antioxidant activity; onion; antioxidant capacity; cyclic voltammetry; spectrophotometric methods; HPLC

INTRODUCTION

Epidemiological studies suggest a protective effect of vegetables and fruits against cancer and coronary heart disease. An attractive hypothesis is that vegetables and fruits contain compounds that have a protective effect, independent of that of known nutrients and micronutrients. Plant polyphenols, a large group of natural antioxidants ubiquitous in a diet in vegetables and fruits, have considerable interest as phytoprotectants that may serve to prevent or delay the onset or development of degenerative diseases (*1*). Among them, the dietary flavonoids and their antioxidant activity have attracted

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much attention in relation to their possible role in the prevention of oxidative stress induced by chronic diseases (*2*). In this context, the onion is an interesting vegetable plant species due to its natural high content of flavonoids and its widespread popularity all over the world (*3*). Onion ranked highest in quercetin (Q) content in a survey of 28 vegetables and nine fruits (*4*). The amount of Q in onions varies with bulb color and type and is distributed mostly in the outer skins and rings (*5, 6*). In fresh vegetables, mainly glycosylated flavonols and other flavonoids are present but aglycones may be found as a result of food processing (*7*).

The total Q concentration in onion, expressed as Q aglicone, is from traces in white varieties to 2.5–3 mmol/kg of fresh weight in red varieties, where it occurs as various *O*-*β*-glycosides with D-glucose as the main sugar residue. Wiczkowski et al. (*8*) showed that Q in onions exists in four predominat forms: Q aglycone, quercetin-3,4′-di-*O*-*β*-glucoside (Q3,4′G), quercetin-3-*O*-*β*-glucoside (Q3G), and quercetin-4′-*O*-*β*-glucoside (Q4′G), although several forms of the diglucoside and monoglucoside conjugates have been reported in smaller amounts (*9*).

Various model systems have been applied to measure the antioxidant capacity in foods and dietary supplements. In numerous papers dealing with the measurement of the antioxidant capacity, very rarely, a single method was applied. Moreover, the number of methods to measure the antioxidant capacity of foods, nutraceuticals, and other dietary supplements has increased considerably. From evaluation of data presented at the First International Congress on Antioxidants Methods in 2004 as well as consideration of potential end uses of antioxidants, it was proposed that procedures and applications for three assays be considered for standardization: the oxygen radical absorbance capacity (ORAC) assay, the Folin−Ciocalteu method, and possibly the Trolox equivalent antioxidant capacity (TEAC) assay (*10*). However, recently, it was reported that the food antioxidant capacity determined by chemical methods may underestimate the physiological antioxidant capacity, which originates from the close contact of food with the intestinal lumen (*11*). Recently, a highly attractive, convenient, and especially sensitive cyclic voltammetry (CV) method as an instrumental tool for the evaluation of the antioxidant properties of bioactive compounds and total antioxidant capacity of blood plasma, tissue homogenates, and plant extracts has been developed (*12–14*). A cyclic voltammogram (CV tracing) provides information describing the integral antioxidant capacity without the specific determination of the contribution of each individual component. It is based on the analysis of the anodic current (AC) waveform, which is a function of the reductive potential of a given compound in the sample and/or a mixture of components. The total antioxidant capacity of the sample is a function combining two sets of parameters. The first parameter is the biological oxidation potential, whereas the second parameter is the intensity of the AC (Ia), reflecting the concentration of the components. It has been proposed that the area under the AC wave (related to the total charge) rather than Ia is a better parameter reflecting the antioxidant capacity of the sample (*12, 13*). CV is a convenient technique for studying the electrochemical behavior of electroactive substances. CV allows the determination of the antioxidant activity of electroactive compounds as well as the total antioxidant activity of edible plants before ingestion. The electrochemical behavior of Q has been almost always studied in organic solvents due to constraints imposed by its solubility characteristics (*14*), but no information exists with respect to Q glucosides. Therefore, the aim of this work was to show an application of CV, TEAC, and peroxyl radical trapping capacity (PRTC) spectrophotometric assays for the determination of the relative contribution of Q and its glucosides to the antioxidant capacity of onions. For this purpose, for the first time, the antioxidant activities of quercetion and its glucosides were measured by CV, TEAC, and PRTC methods. Moreover, the onion flavonoids profile was provided by high-performance liquid chromatography−mass spectrometry (HPLC-MS) method, and then, the qualitative data were used for the calculation of relative contributions of Q and its glucosides to the total antioxidant capacity of onion extracts.

MATERIALS AND METHODS

Reagents. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2′-azobis (2-amidopropane) dihydrochloride (ABAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO). Myoglobin from horse heart and hydrogen peroxide (ACS reagent, 30 wt % solution in water) were obtained from Sigma-Aldrich (Sigma-Aldrich Chemie, Steinheim, Germany). Q and Q4′G were from Extrasynthese (Genay, France), Q3G was from Fluka, and Q3,4′G was from Polyphenols Laboratorie AS (Norway). Standards were dissolved in 80% methanol, and their concentrations were confirmed by UV measurement. Methanol, acetonitrile, formic acid, acetic acid (supra-gradient), and sodium acetate were from Merck KGaA (Darmstadt, Germany). ACW (antioxidant capacity of water-soluble substances) and ACL (antioxidant capacity of lipid-soluble substances) kits (no. 400.801) for PCL assay were from Analytik Jena AG (Jena, Germany). All other reagents of reagent-grade quality were from POCh (Gliwice, Poland). Water was purified with a Milli-Q-system (Milipore, Bedford, MA).

Sample Preparation. Flesh scales of two onion varieties, Sochaczewska (*Allium cepa* sp.) and Szalotka 1902 (*Allium cepa* var. *aggregatum*), kindly provided by the Research Institute of Vegetable Crops in Skierniewice (Poland), were utilized as materials for analysis. Sochaczewska is a typical onion with a yellow-brown bulb color, while Szalotka 1902 belongs to shallot onions characterized by approximately four times smaller bulbs when compared to Sochaczewska bulbs. The tops and bottoms of the onion bulbs, outer dry skins, and any inedible outer portions were removed. Then, onion flesh scales were cut, frozen, and lyophilized, and then, lyophilized samples were stored at –80 °C prior to further preparation. The dry matter contents of Szalotka 1902 and Sochaczewska were 14.3 and 19.8%, respectively. For CV experiments, approximately 75 mg of lyophilized onion tissue was extracted with 1 mL of 80% methanol by 30 s of sonication. Next, the mixture was vortexed for 30 s, again sonicated and vortexed, and centrifuged for 5 min (5000*g* at 4 °C). That step was repeated five times, and the supernatants were collected in a 5 mL flask. For spectrophotometric experiments and HPLC analysis, approximately 150 mg of lyophilized onion tissue was extracted with 1 mL of 80% methanol by 30 s of sonication. Next, the mixture was vortexed for 30 s, again sonicated and vortexed, and centrifuged for 5 min (5000*g* at 4 °C). That step was repeated five times, and the supernatants were collected in a 5 mL flask. The use of a twice lower onion extract concentration for CV measurements than those for spectrophotometric experiments and HPLC analysis was dictated to avoid unnecessary adsorption of oxidation products of flavonoids on the surface of the glassy carbon electrode during analysis as recently was described (*14–17*).

Analysis of Q and Its Main Glycosides by HPLC-Ultraviolet (UV)-MS. Identification and quantitative analysis of Q and its selected glycosides were performed in 80% methanol onion extracts. Separation was conducted by means of a HPLC-UV method in a gradient system, and identification was performed by means of standards and determination of molecular ions of selected compounds on a MS combined with HPLC-UV (*8*). The analyses were carried out on Shimadzu HPLC system (Shimadzu, Kyoto, Japan) consisting of two pumps (LC-10 AD and LC-10 AD_{VP}), an UV detector (SPD-10 A) set at 360 nm, a MS detector (QP8000α), an autosampler set to 5 μL injection (SIL-10

AD_{VP}), and a column oven (CTO-10 AS_{VP}), and a system controller (SCL-10 A_{VP}) was used for the validation of the results. HPLC-UV-MS analysis was performed at 35 °C with a flow rate of 0.2 mL/min on a C18(2) Luna 3 μm column, 150 mm × 2 mm (Phenomenex, Torrance, CA). The flavonoids were eluted in a gradient system composed of water with 0.05% formic acid, pH 2.9 (solvent A), and acetonitrile (solvent B). Gradients were as follows: 17–80–80–17–17% B at gradient time, t_G = 0–17–27–28–55 min. Confirmation of the analytes identity was done by the mass spectrometer with the following parameters: CDL temperature, 240 °C; CDL voltage, −50 V; probe voltage, −3.5 kV; nebulizer gas, N_2; flow, 2.8 mL/min; and defragmentation voltage, −45 V.

Voltammetric Experiments. A potentiostat/galvanostat KSP system (Poland) was used for voltammetric experiments. A conventional three-electrode system, (i) a 3 mm diameter glassy carbon electrode working electrode (BAS MF-2012), (ii) a Ag/AgCl electrode as a reference electrode, and (iii) a platinum electrode as a counterelectrode, was used. Cyclic voltammetric experiments were performed with a solution of standard/extracts mixed with 0.1 M sodium acetate-acetic buffer (pH 5.5) at a ratio of 1:1 (v/v) in 80% methanol according to Cosio et al. (*18*). The sodium acetate-acetic buffer also acted as a supporting electrolyte for the CV measurements. The voltammetric experiments were performed at room temperature using an apparatus cell (volume, 200 μL), to which analyzed standard/extract solutions mixed with the buffer solution were introduced. Exactly 100 μL of the extract/standard and 100 μL of buffer solution were used. The cyclic voltammograms were acquired in the range of –100 to +1300 mV at a scanning rate of 100 mV s^{-1} at 2 mV intervals. Prior to use, the surface of the glassy carbon electrode was carefully polished with 0.05 μm alumina paste and ultrasonically rinsed in deionized water and after that washed with methanol. This procedure was repeated after each cycle. For the test purpose, the total charge below the anodic wave curve of the voltammogram was measured. The CV method was actually based on the correlation between the total charge below the anodic wave of cyclic voltammograms and the antioxidant capacity of the sample and reference substance. The 80% methanol solution of Trolox within the concentration range of 0.025–1.25 mM was used, and the results were expressed as μmol Trolox/g dry matter (dm). The total charge under the anodic wave of the background signal (solvent + supporting electrode) was subtracted from total charge under the anodic wave obtained for each standard, and the sample was measured within the range of +100 to +1100 mV.

Measurement of the Antioxidant Activity of Q and Its Glucosides by TEAC Assay. This method was used to determine the antioxidant activity of Q and its glucosides as was described by Miller and Rice-Evans (*19*). This method was based on the inhibition by standards of these compounds of the absorbance of the radical cation of ABTS ($ABTS^{\bullet+}$), which has a characteristic long-wavelength absorption spectrum showing maxima at 660, 734, and 820 nm. The $ABTS^{\bullet+}$ radical cation is formed by the interaction of ABTS (150 μM) with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin (2.5 μM) with H_2O_2 (75 μM). Antioxidant compounds suppress the absorbance of the $ABTS^{\bullet+}$ radical cation to an extent and on a time scale dependent on the antioxidant capacity of the substance under investigation. In this study, the analytical strategy based on the lag phase measurement at 734 nm was used as it was described in detail by Rice-Evans and Miller (*20*). The standard curve based on the length of time of the lag phase vs Trolox concentration was constructed within the range of 0.05–1.25 mM Trolox. The measurements were carried at 30 °C using a temperature-controlled spectrophotometer UV-160 1PC with CPS-Controller (Shimadzu, Japan).

Measurement of the Antioxidant Activity of Q and Its Glucosides by PRTC Assay. A simple method of determining the PRTC based on the use of ABAP decomposition as a free radical source and the use of ABTS oxidation as the reaction indicator was employed to express the antioxidant activity of compounds under investigation as described Bartosz et al. (*21*). Briefly, 0.1 M sodium phosphate buffer, pH 7.0, was preheated to 37 °C and added to a cuvette so as to obtain 3 mL of the final reaction volume. Then, 90 μL of 5 mM ABTS solution and standard compound or Trolox solution was added followed by 300 μL of 200 mM ABAP solution. The cuvettes were placed in a thermostatted recording spectrophotometer UV-160 1PC with a CPS-Controller (Shimadzu, Japan) adjusted to 37 °C inside the cuvettes, and the induction time of ABTS oxidation as a function of the concentration of Trolox or standard compounds in the reaction mixture was measured at 414 nm. The standard curve based on the induction time of ABTS oxidation vs Trolox concentration was constructed within the range of 0.05–1.25 mM Trolox.

Determination of TEAC and PRTC of Onion Extracts. TEAC was determined following a procedure described by Re et al. (*22*) with a minor modification described below. For measurements, the $ABTS^{\bullet+}$ solution was diluted with 80% methanol to the absorbance of 0.70 ± 0.02 at 734 nm. For the photometric assay, 1.48 mL of the $ABTS^{\bullet+}$ solution and 20 μL of the extracts or Trolox standards were mixed and then measured immediately after 6 min at 734 nm at 30 °C using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). Appropriate solvent blanks were run in each assay. The TEAC of 80% methanol extracts was calculated, using Trolox standard curve, on the basis of reduction in the absorbance of the $ABTS^{\bullet+}$ solution at 734 nm. The 80% methanol solution of Trolox within the concentration range of 0.05–1.25 mM was used for construction of the calibration curve. The PRTC of onion extracts was determined as described previously for flavonoid standards.

Determination of DPPH Radical Scavenging Activity (RSA) of Onion Extracts. The $DPPH^{\bullet}$ scavenging activity was determined using an 80% methanol extract of onion as described previously in detail (*23*). The Trolox standard solutions (concentration 0.1–2.0 mM) in 80% methanol were assayed under the same conditions, and then, the $DPPH^{\bullet}$ scavenging activity of the samples was expressed in terms of TEAC on the basis of reduction in the absorbance of the $DPPH^{\bullet}$ solution by standards at 515 nm.

Determination of ACW and ACL Compounds by Photochemiluminescence (PCL) Method. The PCL method was used to measure the antioxidant activity of onion extracts with a Photochem apparatus (Analytik Jena, Leipzig, Germany) against superoxide anion radicals generated from luminol, a photosensitizer, when exposed to UV light. The light source was a mercury discharge phosphorus-coated lamp with maximal excitation at λ = 351 nm. The antioxidant activity of 80% methanol extracts of onions was measured using both "ACW" and "ACL" kits provided by the manufacturer designed to measure the antioxidant activity of hydrophilic and lipophillic compounds, respectively. The assay was carried out as previously described (*24*).

Measurement of Folin−Ciocalteu Reagent (FCR) Reducing Capacity of Onion Extracts. The FCR reducing capacity of onion extracts by the means of FCR application was carried out according to Shahidi and Naczk (*25*). Briefly, 0.25 mL of 80% methanol extract was mixed with 0.25 mL of FCR previously diluted with distilled water (1:1 v/v), 0.5 mL of saturated sodium carbonate (Na_2CO_3), and 4 mL of water. The mixture was incubated at room temperature for 25 min and centrifuged at 2000*g* for 10 min. The supernatant absorbance was measured at 725 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). The data were expressed as mg of Q4′G equivalents on dry matter basis.

Determination of the Relative Contribution of Q and Its Glucosides to the Antioxidant Capacity of Onions. The contributions of Q, Q3G, Q4′G, and Q3,4′G based on antioxidant activities provided by spectrophotometric methods (TEAC and PRTC) as compared to the antioxidant capacity of two onion varieties obtained by the same spectrophotometric and CV methods were calculated. The following point of view was taken into account when the contributions of the individual compounds to the antioxidant capacity of the samples were calculated. At first, the mean content of investigated compounds, provided by HPLC analyses, was expressed as micromoles of individual compound per gram of dry matter and then multiplied by their antioxidant activities provided by TEAC and PRTC methods. After that, the result for each compound contribution was divided by the antioxidant capacity of onion extract determined by the respective assay and finally expressed as percentage of contribution.

Statistical Analysis Method. The results are given as the means and the standard deviations of three independent experiments. Statistical analysis was performed using Student's *t* test, and the significance level was set at $P < 0.05$.

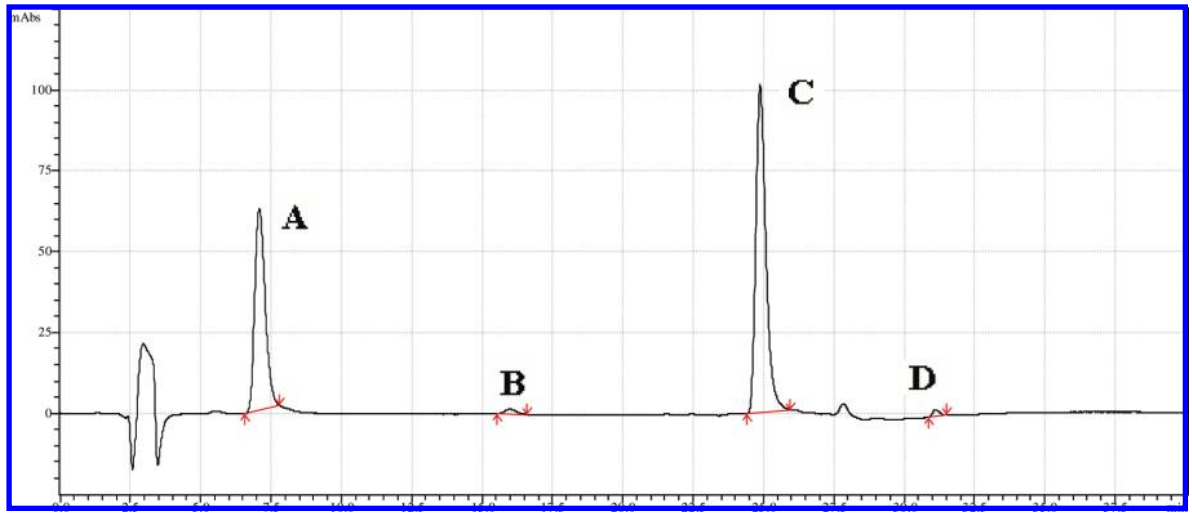

Figure 1. HPLC-UV chromatogram of onion flavonoids recorded at 360 nm. The concentration of the onion extract was 30 mg mL^{-1} in 80% methanol. The operative conditions were as described in the Materials and Methods. Peak identification: A, Q3,4′G; B, Q3G; C, Q4′G; and D, Q.

Table 1. Contents of Q, Q3G, Q4′G, and Q3,4′G in Onions[a]

	μmol/g dm				
onion variety	Q	Q3G	Q4′G	Q3,4′G	total
Sochaczewska	0.14 ± 0.01	0.19 ± 0.01	8.00 ± 0.37	4.82 ± 0.20	13.15 ± 0.56
Szalotka	0.14 ± 0.01	0.21 ± 0.01	10.49 ± 0.08	8.57 ± 0.03	19.41 ± 0.10

[a] Data are expressed as means ± standard deviations ($n = 3$).

RESULTS AND DISCUSSION

Analysis of Q and Its Main Glycosides by HPLC-MS. The data provided by HPLC-UV-MS showed the presence of Q and its glucosides, namely, Q3,4′G, Q3G, and Q4′G, in 80% methanol extracts of lyophilized onion bulbs (**Figure 1**). The HPLC-UV identification of flavonoids in onion extract was confirmed by ESI-SIM (electrospray ionization-selected ion monitoring) spectra based on the following m/z values of negative ions of Q3,4′G (m/z = 625, 463, and 301), Q3G (m/z = 463 and 301), Q4′G (m/z = 463 and 301), and Q (m/z = 301) (*26*). Dominant forms of Q in the onion var. Sochaczewska and Szalotka included Q4′G (61 and 54%), Q3,4′G (37 and 44%), Q3G (1.4 and 1.1%), and free Q (1.1 and 0.7%), respectively (**Table 1**).

Antioxidant Activity of Q and Its Main Glucosides Provided by CV Method. In this study, cyclic voltammograms of examined compounds were recorded. Cyclic voltammograms of 0.1 mM solutions of Q, Q3G, Q4′G, and Q3,4′G in 50 mM acetate-acetic buffer (pH 5.5) in 80% methanol recorded with a glassy carbon electrode (vs Ag/AgCl) scanned at 100 mV s^{-1} are shown in **Figure 2a**−**d**. For these voltammograms, the anodic peak potential, E_a, was observed at 310, 390, 482, and 800 mV for Q, Q3G, Q4′G, and Q3,4′G, respectively. Q and Q3G exhibit almost reversible oxidation steps (**Figure 2a**,**b**) while Q4′G and Q3,4′G are irreversible species (**Figure 2c**,**d**). This behavior, which is in relation to their molecular structures, clearly indicates the importance of the 3′,4′-dihydroxy substitute on the B ring of the Q molecule for its electrochemical activity. The observed anodic wave showed well-resolved peaks and a shoulder in the potential region up to 1.1 V. Similarly, voltammograms obtained for the standard solutions of Trolox (0.05–1.25 mM) also showed well-resolved peaks in the potential region up to 1.1 V. A typical CV tracing of different Trolox concentrations is shown in **Figure 3**. The total charge under the AC waveform, provided by CV computer software, was used to calculate the reducing activity of the compound under investigation, based on the function AC vs set of Trolox solutions (**Figure 4**) as it was suggested by Chevion et al. (*12*) and Martinez et al. (*27*). The reducing activity provided by CV method was equal to the millimolar concentration of Trolox solution having the antioxidant capacity equivalent to a 1.0 mM solution of the substance under investigation. The experiment showed the highest reducing activity of Q, while the Q3G, Q4′G, and Q3,4′G exhibited about 66, 51, and 27% of the reducing power noted for Q. The data are compiled in **Table 2**.

Antioxidant Activity of Q and Its Main Glucosides Provided by TEAC and PRTC Methods. In this study, the antioxidant activity of Q and its main glucosides obtained from CV measurements was compared with TEAC and PRTC of Q, Q3,4′G, Q3G, and Q4′G provided by spectrophotometric assays. Both TEAC and PRTC are equal to the millimolar concentration of a Trolox solution having the antioxidant capacity equivalent to a 1.0 mM solution of the substance under investigation. The analytical strategy of TEAC was based on the lag phase measurement at 734 nm showing significant differences in the behavior of these compounds under the assay. The studies gave evidence that the highest lag phase was for Q while the lowest one was for Q3,4′G. The order of the antioxidant activity was as follows, Q > Q3G > Q4′G > Q3,4′G, as was shown in **Figure 5**. The antioxidant activity of Q was in excellent agreement to that reported by Rice-Evans et al. (*28*); however, the antioxidant activities of Q glucosides under this assay are reported for the first time. The data provided by TEAC assay were confirmed by the PRTC method; however, the antioxidant activities of Q3G and Q4′G were lower by 25 and 75%, respectively (**Figure 5**). Q3,4′G showed no antioxidant activity under both applied spectrophotometric assays. In contrast, Q3,4′G exhibited low activity under the CV experiment. The order of the antioxidant activity of Q and its main glucosides provided by CV, TEAC, and PRTC methods was in accordance to the earlier report of Ioku et al. (*29*) where peroxyl radical-scavenging activities of these compounds were examined in

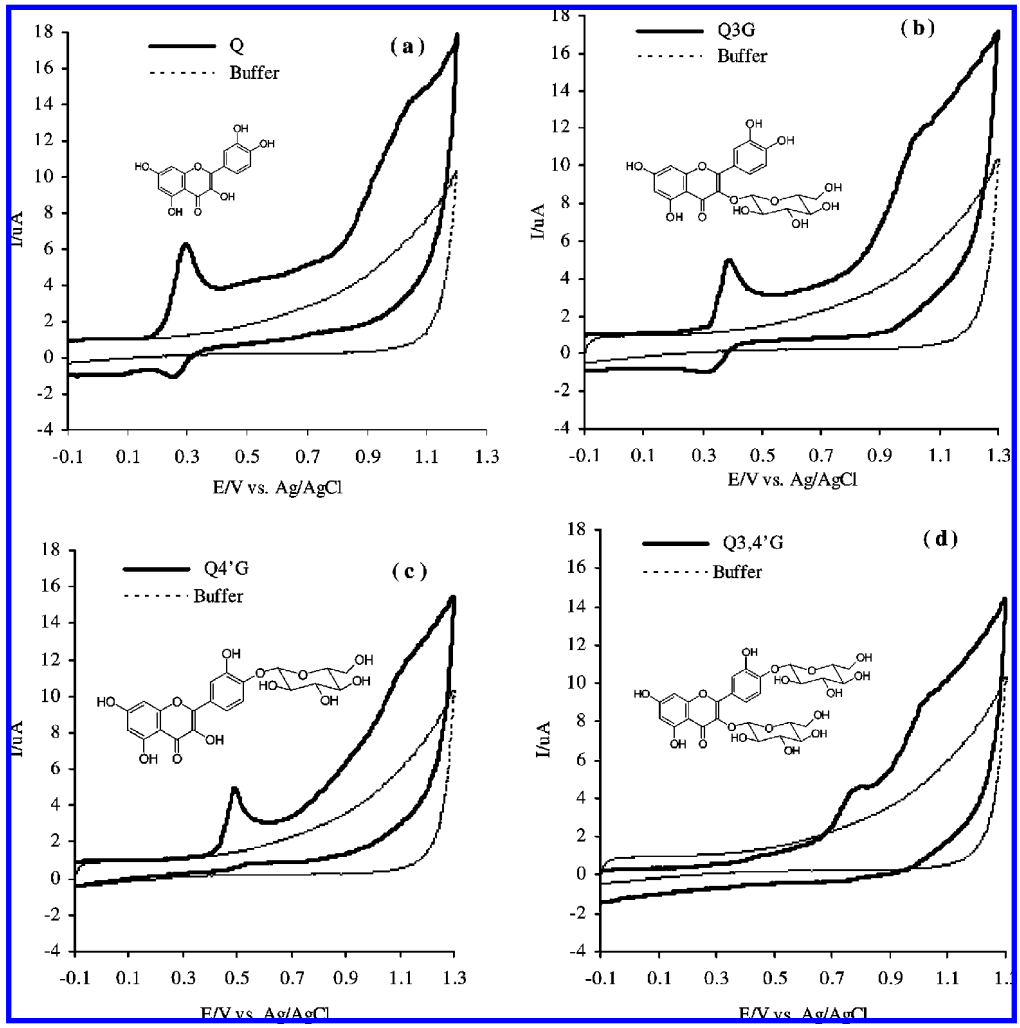

Figure 2. Cyclic voltammograms of 0.1 mM standard solutions of (**a**) Q, (**b**) Q3G, (**c**) Q4′G, and (**d**) Q3,4′G in 50 mM sodium acetate-acetic buffer (pH 5.5) in 80% methanol; scan rate, 100 mV s^{-1}.

solution and liposomal phospholipid suspensions when phospholipid bilayers were exposed to aqueous oxygen radicals. In our study, the antioxidant activity of Q and Q3G provided by TEAC and PRTC assays was higher when compared to that obtained from CV experiments. In contrast, the reducing activity of Q4′G provided by the CV method (**Table 2**) was almost 3-fold and six times higher to that from TEAC and PRTC assays (**Figure 5**), respectively. These results clearly indicate that the antioxidant properties of Q glucosides are the most affected by a free hydroxyl group forming a catecholic set in ring B of these compounds. This conclusion was supported by a recent study in structure–activity relationship (SAR) of flavonoids (*30*). Currently, it is well-recognized that the structural features and nature of substitutions on rings B and C determine the antioxidant activity of flavonoids. It includes (i) the degree of hydroxylation and the positions of the OH groups in the B ring; (ii) the presence of hydroxyl groups at the 3′-, 4′-, and 5′-positions of ring B, which has been reported to enhance the antioxidant activity of flavonoids as compared to those that have a single hydroxyl groups; (iii) a double bond between C-2 and C-3, conjugated with the 4-oxo group in ring C, which enhances the radical-scavenging capacity of flavonoids; and (iv) a double bond between C-2 and C-3, combined with a 3-OH, in ring C, which also enhances the active radical scavenging capacity of flavonoids (*28, 30*). It was also reported that substitution of the 3-OH results in an increase in the torsion angle and loss of coplanarity and, subsequently, reduced antioxidant activity, while substitution of hydroxyl groups in ring B by methoxyl groups alters the redox potential, which affects the free RSA of flavonoids (*31*).

Reducing Capacity of Onions Derived from Voltammetric Experiments. Cyclic voltammograms of analyzed 80% methanol extract of onions were recorded as shown in **Figure 6**. The observed anodic wave showed a well-resolved peak identified as Q4′G and a broadened wave related to Q, Q3G, and Q3,4′G. All tested compounds previously exhibited different oxidation potentials. Therefore, the total charge under the AC waveform, provided by CV computer software, was used to calculate the antioxidant capacity of the sample, based on the function AC vs set of Trolox solutions. The data showed about 12% higher antioxidant capacity of onion var. Sochaczewska than onion var. Szalotka, although these results were not significantly different (**Table 3**).

Antioxidant Capacity of Onions Derived from TEAC, PRTC, DPPH RSA, FCR, and PCL Assays. The following procedures and applications of three assays can be considered for standardization of antioxidant capacity measurements: the Folin−Ciocalteu method, the ORAC assay, and possibly the TEAC assay (*10*). In this study, two from the above-listed spectrophotometric assays were taken for the evaluation of antioxidant capacity of onions. Moreover, two additional spectrophotometric methods, namely, PRTC and DPPH RSA, and one based on PCL, were used to get a full screen of antioxidant capacities provided by different methods. Therefore,

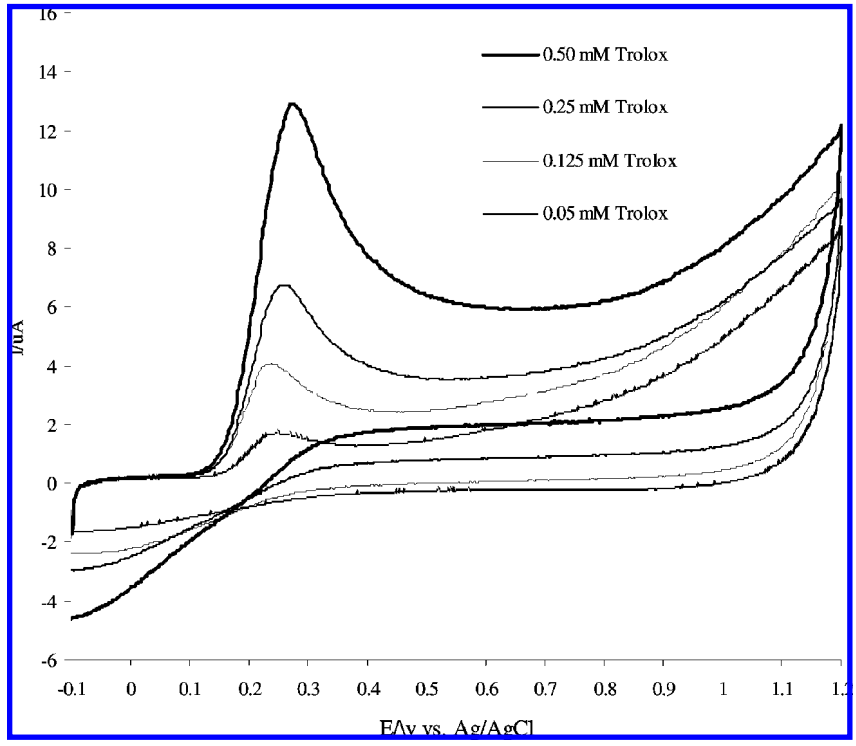

Figure 3. Selected cyclic voltammograms of Trolox concentrations within the range of 0.05–1.25 mM in 50 mM sodium acetate-acetic buffer (pH 5.5) in 80% methanol; scan rate, 100 mV s^{-1}.

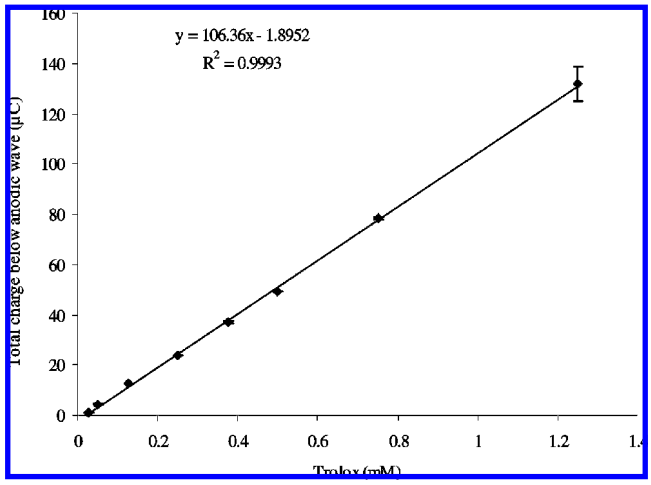

Figure 4. Dependency of the total charge under the anodic wave as a function of increasing concentration of Trolox (0.05–1.25 mM). The total charge below the anodic wave of the background signal (solvent + supporting electrolyte) was subtracted from the total charge obtained for each Trolox concentration within the range of 100–1100 mV.

the 80% methanol extracts of onion were examined for their free RSA against $ABTS^{\bullet+}$ cation radical (TEAC), peroxyl radicals ($ROO^{\bullet}$), $DPPH^{\bullet}$ radicals, superoxide anion radicals ($O_2^{\bullet-}$) generated from luminol (PCL), and their reducing capacity by the means of FCR application. The data are compiled in **Table 4**. The antioxidant capacity of onions determined by CV method was twice higher than PCL ACW, about 50% higher than PRTC, DPPH RSA, and the converted FCR reducing capacity values, but it was 3-fold and about four times lower when compared to TEAC and PCL ACL, respectively. The increased order of the antioxidant capacity of onions was PCL ACW < FCR < DPPH RSA = PRTC < CV < TEAC < PCL ACL. The highest antioxidant capacity of onion was found under cumulative consideration of PCL (ACW + ACL)

Table 2. Reducing Activity of Q, Q3G, Q4′G, and Q3,4′G Provided by the Cyclic Voltammetric Method ($n = 3$)[a]

compound	total charge below anodic wave[b] (μC)	reducing activity (mM Trolox)
Q	37.78 ± 2.12	3.92 ± 0.20
Q3G	25.64 ± 0.42	2.59 ± 0.04
Q4′G	19.28 ± 0.97	1.99 ± 0.09
Q3,4′G	9.47 ± 0.49	1.07 ± 0.05

[a] Standards (100 μM) were dissolved in 80% methanol. [b] The total charge below the anodic wave of the background signal (solvent + supporting electrolyte) was subtracted from the total charge obtained for each compound under investigation within the range of 100–1100 mV.

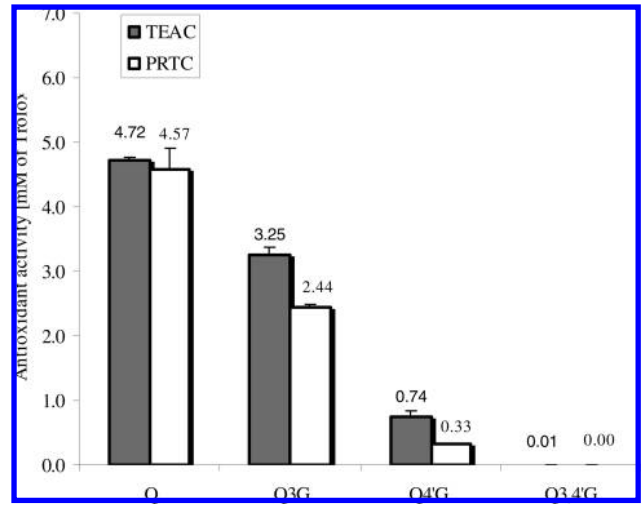

Figure 5. Antioxidant activity of Q, Q3,4′G, Q3G, and Q4′G provided by TEAC and PRTC assays ($n = 3$).

and TEAC results. In this study, an interesting finding was that the antioxidant capacity of onions determined by CV method was about 50% higher than the converted FCR reducing

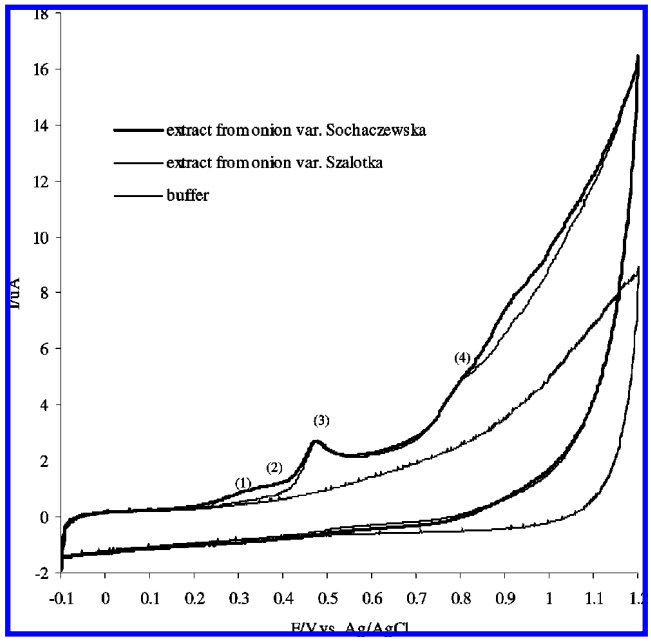

Figure 6. Cyclic voltammograms of onion extract (15 mg/mL 80% methanol) in 50 mM acetate-acetic buffer (pH 5.5) in 80% methanol; scan rate, 100 mV s^{-1}. Peak identification: 1, Q; 2, Q3G; 3, Q4′G; and 4, Q3,4′G.

Table 3. Reducing Capacity of Onions Measured by the Cyclic Voltammetric Method[a]

onion variety	total charge below anodic wave (μC)[b]	reducing capacity (μmol Trolox/g dm)
Sochaczewska	15.38 ± 1.23 a	10.83 ± 0.77 a
Szalotka 1902	13.57 ± 1.25 a	9.69 ± 0.78 a

[a] Data are expressed as means ± standard deviations ($n = 3$). Means in a column followed by the same letter are not significantly different ($p \leq 0.05$). [b] The total charge below the anodic wave of the background signal (solvent + supporting electrolyte) was subtracted from the total charge obtained for each onion extract within the range of 100–1100 mV.

Table 4. TEAC, PRTC, DPPH RSA, ACW and ACL Compounds (PCL = ACW + ACL), and FCR Reducing Capacity of Onions[a]

assay/onion variety	Sochaczewska	Szalotka
TEAC (μmol Trolox/g dm)	30.81 ± 0.18 a	29.48 ± 1.77 a
PRTC (μmol Trolox/g dm)	7.23 ± 0.10 a	5.96 ± 0.40 b
DPPH RSA (μmol Trolox/g dm)	7.86 ± 0.33 a	6.14 ± 1.02 b
PCL ACW (μmol Trolox/g dm)	4.92 ± 0.13 a	4.56 ± 0.14 a
PCL ACL (μmol Trolox/g dm)	42.04 ± 0.19 a	33.68 ± 1.49 b
PCL (ACW + ACL)	46.96 ± 0.17 a	38.24 ± 1.24 b
FCR reducing capacity (mg Q4′G equiv/g dm)	1.35 ± 0.01 a	1.40 ± 0.02 a
(μmol Trolox/g dm)	(5.80 ± 0.05)[b]	(5.98 ± 0.10)[b]

[a] Data are expressed as means ± standard deviations ($n = 3$). Means in a row followed by the same letter are not significantly different ($p \leq 0.05$). [b] Values indicated in the brackets show the converted FCR reducing capacity into antioxidant capacity expressed as μmol Trolox/g dm when the antioxidant activity of Q4′G equal to 1.99 mM was obtained from the CV experiment.

capacity. It was in accordance with selection of Q4′G as a reference compound in the FCR reducing capacity assay. This compound was found in the onion var. Sochaczewska and Szalotka as a dominant one, forming about 61 and 54% of analyzed forms of Q. Therefore, because Q3,4′G also contributed significantly to the content of remaining forms of Q in onion, it can be suggested that the antioxidant capacity measured by the CV method strongly corresponds to the reducing power of both Q4′G and Q3,4′G. Therefore, it can be concluded that CV, PRTC, DPPH RSA, and FCR reducing capacity, when dominant in onion Q glucoside is taken as a reference compound, can be recommended as appropriate methods for evaluation the antioxidant capacity of onions.

Table 5. Relative Contribution of Q, Q3G, Q4′G, and Q3,4′G to the Antioxidant Capacity of Onions (%)

onion variety/assay	relative contribution to the antioxidant capacity: Q (%)	Q3G (%)	Q4′G (%)	Q3,4′G (%)	total contribution (%)
Sochaczewska					
CV	6.1	5.7	54.7	0.5	67.0
TEAC	2.1	2.0	19.2	0.2	23.5
PRTC	8.9	6.4	36.5	0	51.8
Szalotka					
CV	6.8	7.0	80.1	0.9	94.8
TEAC	2.2	2.3	26.3	0.3	31.1
PRTC	10.7	8.6	58.1	0	77.4

Determination of the Relative Contribution of Q and Its Glucosides to the Antioxidant Capacity of Onions. The relative contributions of Q, Q3G, Q4′G, and Q3,4′G to the antioxidant capacity of onions determined by CV, TEAC, and PRTC methods are shown in **Table 5**. The calculated contribution was based on the assumption that no interaction occurred among Q and its glucosides in onions. Measurement of individual contents of Q and its glucosides in onions, together with their individual antioxidant activity determined by CV, TEAC, and PRTC assays, may indicate the extent to which the antioxidant screen of onions is accounted for and, hence, reveal the activity of unidentified antioxidants (antioxidant gap). The results showed a low contribution of Q, Q3G, and Q3,4′G to the antioxidant capacity of onions derived from CV, TEAC, and PRTC assays, while the highest contribution to the antioxidant capacity of onions was provided by Q4′G. It was found that the contribution of Q4′G to the total antioxidant capacity of onions was the highest when the CV method was used, followed by PRTC and TEAC methods. This finding also indicates the specificity of the rank of methods used as CV > PRTC > TEAC since the lowest antioxidant gap was provided by CV while the highest one was provided by the TEAC method.

Moreover, the provided contribution as well as antioxidant and reducing activities of onion Q glucosides indicate different redox properties of Q4′G and Q3,4′G since these compounds exhibit almost irreversible oxidation steps (**Figure 2c**,**d**). In this work, an additional oxidation process resulted in a weak-resolved peak within the region of 900–1000 mV (**Figures 2a**−**d** and **6**). This peak can be explained by the oxidation of byproduct formed in the preceding electrochemical steps. The similar observation for Q was reported by Timbola et al. (*14*). Therefore, there is a possibility of adsorption of the oxidation products of Q4′G and Q3,4′G on the carbon electrode surface, resulting in lower reducing capacity of the onion samples (*15*). Moreover, the variation in media, substrates, oxidants, evaluating indices, and detecting methods is known to greatly influence the behavior of antioxidants and their evaluation in different systems, which may account for the data presented in this study.

There is still a controversy as to whether the electrochemical assay measures exactly the antioxidant activity or reducing power (*32*). In this study, the antioxidant capacity of onions derived by CV method was more than 50% higher than the converted FCR reducing capacity.

This finding clearly indicates the CV method as a suitable method for the determination of the reducing capacity based

on the electrochemical properties of compounds under the test. Recently, the CV was shown as an efficient instrumental tool for evaluating the antioxidant properties of low molecular weight antioxidants in human plasma, animal tissues, edible plants, wines, different types of tea and coffee (*12, 13, 24, 33–35*), and pseudo cereal-based products (*23*). Therefore, it can be suggested that the CV assay is an efficient tool for describing the reducing activity of Q and its glucosides based on their redox properties; however, care should be taken during analytical work to avoid the adsorption of oxidative products on the carbon electrode surface.

Concluding Remarks. The use of CV and spectrophotometric methods based on free radical-scavenging activities (TEAC and PRTC) and reducing power (FCR) was applicable for the evaluation of the antioxidant activity of Q and its glucosides, the antioxidant capacity of onion, and then the contribution of the individual compounds to the antioxidant capacity, followed by qualitative and quantitative analysis of these compounds.

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