Electrochemical Determination of Ascorbigen in Sauerkrauts

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Abstract Ascorbigen (ABG), 2-*C*-(indol-3-yl)methyl-α-L-*xylo*-hex-3-ulofuranosono-1,4-lactone, was synthesised and its structure and purity was confirmed by means of ^{1}H NMR and ^{13}C NMR spectra and HPLC-PDA, respectively. The electrochemical behaviour of ABG was studied by cyclic voltammetry (CV) method for pH within the range of 3.0–7.0 and further characterisation was performed by differential pulse voltammetry (DPV) at pH 5.0. Voltammetric studies of ABG at glassy carbon electrode showed one irreversible oxidation peak (centred at E_p=0.950 V for pH 5.0). The anodic peak current potential related to the irreversible oxidation of ABG was shifted towards more positive potentials with decreasing pH values. The linear response between concentration of ABG within the range of 0.08–0.75 mM and recorded current was observed. The DPV method was shown to be more sensitive when compared to the CV method. ABG showed a significant reducing activity provided by CV, whilst the antioxidant activity of ABG against $O_2^{-\bullet}$ was negligible. Electrochemical behaviour of ABG standard was applied successfully for the quantification of natural ABG in sauerkrauts by DPV and HPLC-CoulArray methods. Based on the developed analytical methods, ABG content increased during natural fermentation of cabbage at 0.5% and 1.5% NaCl levels, and results were comparable with those found in the literature.

Keywords Ascorbigen · Cyclic voltammetry · Differential pulse voltammetry · HPLC-CoulArray · Antioxidant activity · Sauerkraut

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Introduction

Ascorbigen (ABG) is a natural breakdown product of glucosinolates (GLS) that is found in plants belonging to the *Cruciferae* family and naturally occurs in *Brassica* vegetables, mainly during fermentation of white cabbage (Wagner and Rimbach 2009). Epidemiological studies have shown that frequent *Brassica* vegetable consumption is associated with a reduced cancer risk (Zhang et al. 2006; Higdon et al. 2007; Śmiechowska et al. 2008). Their anticarcinogenic effect is probably due to their high amounts of GLS. When the plant tissue is damaged, GLS are broken down by the endogenous enzyme myrosinase (β-thioglucoside glucohydrolase; EC 3.3.3.1) to release a complex variety of breakdown products (Mithen 2001).

GLS are sulphur-containing glucosides which are classified into aliphatic, aromatic and indole GLS depending on whether they originate from aliphatic amino acids, aromatic amino acids or tryptophan. During sauerkraut preparation,

indole GLS glucobrassicin (GB) yields first an unstable isothiocyanate that further degrades with loss of S to form the intermediate indole-3-carbinol (I3C) that spontaneously reacts with L-ascorbic acid forming ABG at acid pH (Zeligs 1998). It has been reported that ABG is not present in intact plant tissues (Preobrazhenskaya et al. 1993) or it is present in low quantities in just shredded cabbage because a certain amount of GB might have been hydrolysed by the endogenous myrosinase as a consequence of cell damage (Ciska and Pathak 2004; Martinez-Villaluenga et al. 2009; Peñas et al. 2010). ABG formation is strongly dependent on the pH and the already formed ABG remains relatively stable at pH 4–5 characteristics of fermented cabbage products (Aleksandrova et al. 1992). ABG is the most abundant indole GLS breakdown product in sauerkraut (Ciska and Pathak 2004; Agerbirk et al. 1998), a plant food product that has been widely consumed for centuries.

Several in vivo studies have revealed the anticarcinogenic properties of ABG (Sepkovic et al. 1994; Stephensen et al. 1999; Bonnesen et al. 2001). ABG is able to induce phase I and II enzymes that are centrally involved in the detoxification of xenobiotics (Wagner and Rimbach 2009; Higdon et al. 2007). Moreover, ABG is a moderate inducer of the phase II enzyme NQO1 (NADPH-quinone oxido-reductase) in both cultured hepatocytes and in vivo (Wagner et al. 2008a). In addition, ABG is thought to be an immunomodulating agent (Preobrazhenskaya et al. 1993) and its usefulness is largely attributed to its ability to support the body's natural metabolism of hormones (Michnovicz and Bradlow 1991). It is likely that some of the biological effects of ABG are mediated by its breakdown to ascorbic acid, a well-known antioxidant (Joshi et al. 2008).

ABG is a dietary supplement under the provisions of the US Dietary Supplement Health and Education Act of 1994 and it has now been recognised as a useful pharmacological substance with a variety of actions both in vitro and in vivo. The physical and chemical properties of ABG have been studied in detail and it has been reported to be relatively nontoxic (Joshi et al. 2008). However, The American Dietetic Association recommends that, whenever possible, people should consume the nutrients they need as foods and not as supplements (Hunt 1996) and, for instance, the consumption of fermented cabbage should be recommended.

During the last decade, normal-phase supercritical fluid chromatography (Buskov et al. 2000), thin-layer chromatography and overpressured layer chromatographic techniques (Kátay et al. 1997) and liquid chromatography/mass spectrometry (Wagner et al. 2008a) have been useful tools for ABG standard characterisation. In food matrices such as sauerkraut, however, one of the most useful methods used has been the high-performance liquid chromatography with UV detection HPLC-PAD (Martinez-Villaluenga et al. 2009; Peñas et al. 2010). Due to the importance of ABG bioactivity, the progress in analytical methods in food is still of scientific interest.

The electrochemical properties of ABG have not been investigated yet. The knowledge of these properties can be useful for further evaluation of the reducing activity of ABG. Due to its chemical structure, ABG can be subjected to complete oxidation–reduction studies. For this purpose, the cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques and electrochemical detection, and specially the coulometric electrode array system, are particularly suitable for the ABG assay with special focus on its analyses in sauerkrauts. The coulometric electrode array detection system (HPLC-CoulArray) is a new method providing high precision, sensitivity and selectivity by independently setting different potentials in each cell of the detector (Zhang et al. 2008). The HPLC-coulometric array method also exhibits enhanced specificity due to the two-dimensional resolution achieved with retention time and the sequentially increasing potentials set at the coulometric electrodes in series (Yang et al. 2006).

Therefore, the following aims have been addressed in this study: (1) to characterise electrochemical behaviour of ABG, (2) to determine the reducing power of ABG by CV method and then to compare it with the antioxidant activity of ABG determined against superoxide anion radical ($O_2^{-\bullet}$) by a photochemiluminescence (PCL) assay and (3) to develop quantitative analytical methods for the determination of ABG in sauerkraut by DPV and HPLC with CoulArray detection.

Materials and Methods

Chemicals and Reagents

L-Ascorbic acid and 3-hydroxymethyl indole and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). The PCL kit for the determination of the antioxidant activity of ABG was from Analytik Jena AG (Jena, Germany). Methanol, acetic acid (supra-gradient) and sodium acetate were from Merck KGaA, Darmstadt, Germany. All other reagents of reagent-grade quality were from POCh, Gliwice, Poland. Water was purified with a Milli-Q system (Milipore, Bedford, USA).

Synthesis and Identification of Ascorbigen

ABG was obtained by the reaction of ascorbic acid and I3C, as shown in Fig. 1. The synthesis was carried out according

Fig. 1 Synthesis of ABG

indole-3-carbinol (I3C)   L-ascorbic acid (AA)   ascorbigen (ABG)
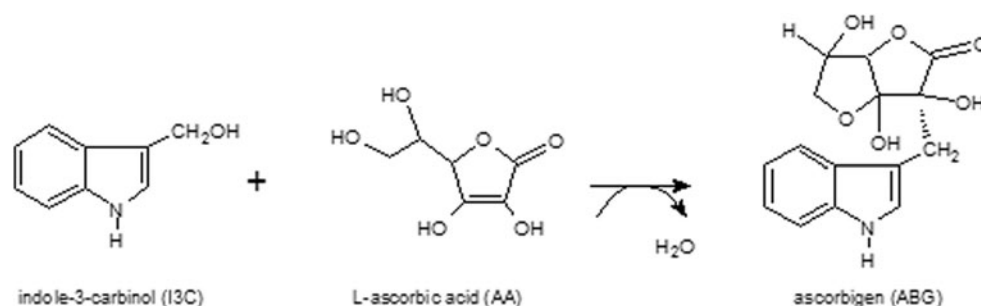
to Kiss and Neukom (1966) with some modifications. Briefly, 440 mg of ascorbic acid (2.5 mM) was dissolved at room temperature in 20 ml of McIlvaine buffer pH 4 and 370 mg of 3-hydroxymethyl indole (2.5 mM) was added. The mixture was kept at room temperature for 1 h, under nitrogen and protected from light. After filtration of solids, the solution was washed with ethyl ether and ethyl acetate. Evaporation of the ethyl acetate extract yielded pure ABG (200 mg), which was kept under nitrogen at −20 °C and protected from light until further analysis. ABG was identified by the ^{1}H NMR and ^{13}C NMR spectra. The ^{1}H NMR and ^{13}C NMR spectra of synthesised ABG were recorded on a Varian Inova Spectrometer operating at 400 and 50 MHz, respectively, using CD_3OD as solvent. Purity of synthesised ABG was performed by HPLC using an Alliance Separation Module 2695 (Waters, Milford, USA) using an ODS-2 column (150×4.6 mm i.d.) (Waters) and a Photodiode Array Detector 996 at 280 nm (Waters, Milford, USA), as in Peñas et al. (2010).

Plant Material and Sauerkraut Production

The fresh white cabbages (*Brassica oleracea* L. ssp. *capitata* cv. Bronco) used in this study were provided by Bejo Iberica S.L. (Madrid, Spain). Fresh cabbage heads were trimmed of outer leaves and their central core was removed. The edible part of the cabbages was then shredded into about 2-mm thick strips using a domestic shredder (Moka Express, Barcelona, Spain). Different salt concentrations (0.5% or 1.5% NaCl) were added onto the shredded cabbage and mixed vigorously. Subsequently, cabbage and brine were transferred to autoclaved polyethylene (8 L; Nalge Nunc International, Rochester, NY, USA). The shredded cabbage was tightly pressed together to exclude air, so the subsequent lactic acid fermentation takes place. Fermentations were performed spontaneously by the autochthonous microbiota present on raw cabbage (natural fermentation). On the third day, cabbage was pricked to remove releasing fermentation gasses. Raw and fermented cabbages were freeze-dried, milled and stored at −20 °C until their analysis.

Procedures

Electrochemical Characterisation of Ascorbigen

Stock solutions of 1 mM ABG in 80% methanol were prepared. A series of ABG standards in 0.1 M ammonium acetate buffer, pH 5.7, were prepared from the ABG stock solution and they were used for the investigation of the electrochemical properties of ABG by CV within the range of 0.05–1.0 mM at pH 4–7 and DPV within the range of 0.0125–0.1 mM at pH 5. The voltammetric experiments were performed with a potentiostat G750 (Gamry Instruments, Warminster, PA, USA). A conventional three-electrode system consisting of a 3-mm diameter glassy carbon working electrode (BAS MF-2012), an Ag/AgCl electrode as a reference electrode and platinum as counter electrode was used in the study.

Determination of the Antioxidant Activity of Ascorbigen Against Superoxide Anion Radical

The PCL method was used to measure the antioxidant activity of ABG against superoxide anion radicals ($O_2^{-\bullet}$) generated from the photosensitiser luminol when it is exposed to UV light. The light source was a phosphorus-coated mercury discharge lamp with maximal excitation at $\lambda=351$ nm. The measurements were carried out with a Photochem® apparatus (Analytik Jena, Leipzig, Germany) according to Zielińska et al. (2007).

Determination of Reducing Power of Ascorbigen by Cyclic Voltammetry

Cyclic voltammetric experiments were performed with ABG standard solutions (100 μM) or Trolox solutions (0.1–2.5 mM) in 0.1 M sodium acetate–acetic buffer (pH 5.0 in 80% methanol) according to Zielińska et al. (2008). The sodium acetate–acetic buffer acted also as a supporting electrolyte for the CV measurements. The cyclic voltammetric experiments were performed at room temperature using an electrochemical cell (200 μl). The cyclic

voltammograms were acquired in the range of −100 to 1,200 mV at a scanning rate of 100 mV s^{-1}. Prior to use, the surface of the glassy carbon electrode was carefully polished with 0.05 μm alumina paste, ultrasonically rinsed in deionised water and then washed with methanol (Cosio et al. 2006). This procedure was repeated after each cycle. The total charge below the anodic wave curve of the ABG voltammogram was calculated. The cyclic voltammograms of Trolox solutions at a concentration range of 0.1–2.5 mM was also acquired in the range of −100 to 1,200 mV, and then the reducing activity of ABG was expressed as Trolox equivalent antioxidant capacity.

Determination of Antioxidant Activity and Reducing Power of Raw and Fermented Cabbages

The PCL and CV methods were used as described for the ABG standard. Extracts from freeze-dried fresh and fermented cabbage were prepared as follows: 100 mg of lyophilised sample was extracted with 1 ml of 80% methanol and then extracted in triplicate by 30 s sonication. Next, the mixture was vortexed for 30 s, again sonicated and vortexed and then centrifuged for 5 min (5,000×g at 4 °C) using a Beckman GS-15 R centrifuge (Beckman Instruments, Inc., Palo Alto, CA, USA). That step was repeated five times and supernatants were collected in 5-ml flask. Final extract concentration was 20 mg/ml.

DPV and HPLC-CoulArray Analysis of Ascorbigen in Fermented Cabbages

Extraction of ABG from raw and fermented cabbage was carried out as described by Peñas et al. (2010). For the DPV
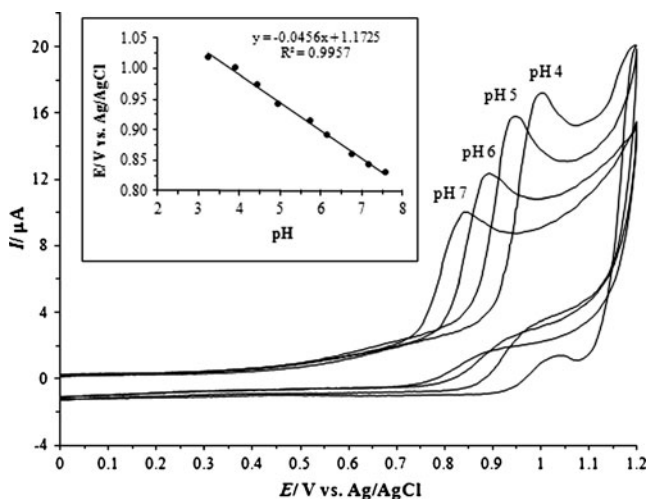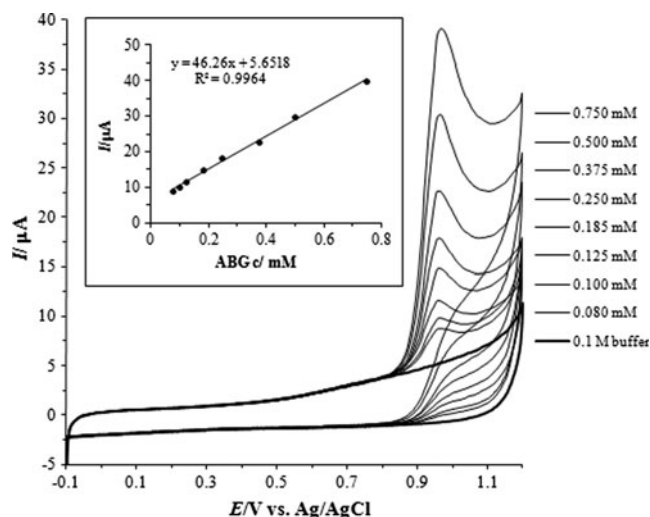

Fig. 3 Selected cyclic voltammograms of ABG within the concentration range of 0.08–0.75 mM at pH 5.0. *Inset* the linear response between the concentration of ABG and anodic peak current

measurements, the residue was dissolved in 80% methanol at a concentration of 200 mg/ml and differential pulse voltammograms were recorded at pH 5.0. The external standard addition method was applied for the identification and quantification of ABG. For the quantification of ABG by the HPLC-CoulArray detector, the residue was dissolved in mobile phase A composition (0.05 M ammonium acetate, pH 5.7 containing 10% acetonitrile). Twenty microlitres of the sample was injected into a Hypersil Gold, 150×4.6 mm i.d., 5 μm size column (Thermo Scientific) at 30 °C. The chro-
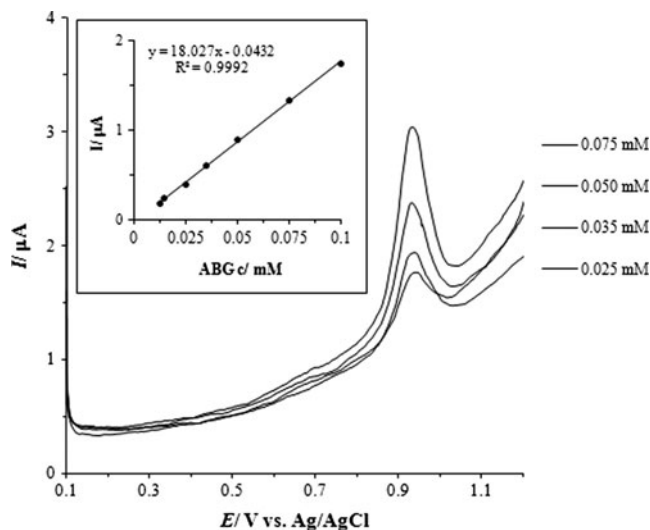

Fig. 2 Cyclic voltammograms of 0.25 mM ABG at pH 4.0, 5.0, 6.0 and 7.0. Cyclic voltammograms were recorded from −100 to +1,200 mV; scan rate, 100 mV s^{-1}. *Inset* the dependence of the anodic peak current potential of ABG vs. pH

Fig. 4 Selected differential pulse voltammograms of different ABG concentrations at pH 5.0. DPVs were recorded from 100 to +1,200 mV; scan rate, 10 mV s^{-1}. *Inset* the linear response between the concentration of ABG and anodic peak current

Table 1 The antioxidant and reducing capacity of ABG standard, raw cabbage and sauerkrauts measured by PCL and CV methods

Material	Antioxidant activity by PCL (μmol Trolox/g d.m.)	Reducing power by CV (μmol Trolox/g d.m.)
ABG standard	0.0128±0.0002a	0.847±0.028a
Raw cabbage	17.86±0.09b	14.36±0.62b
0.5% NaCl sauerkraut	22.28±0.71d	23.01±1.22d
1.5% NaCl sauerkraut	19.01±0.74c	18.81±0.58c

Data are expressed as the means ± standard deviations of three independent determinations. Means in a column followed by different lowercase letters correspond to significant differences ($P \leq 0.05$)

matogram was developed at a flow rate of 1.2 ml/min by eluting in a gradient of mobile phase A (0.05 M ammonium acetate, pH 5.7 containing 10% acetonitrile) and mobile phase B (0.05 M ammonium acetate, pH 5.7 containing 80% acetonitrile) as follows: isocratic 100% A for 1 min, 50% A–50% B linear gradient for 12 min, isocratic 50% A–50% B for 3 min, linear gradient 0% A–100% B for 5 min and isocratic 100% A–0% B for 5 min (to equilibrate to initial conditions). Detection was carried out at 810 mV. The ABG standard was used for identification and quantification by HPLC. The calibration curve was plotted and adjusted by using the method of least squares. The regression coefficient of the ABG curve was >0.990.

Statistical Analysis

Data were subjected to one-way analysis of variation (ANOVA) using Duncan's multiple range test for uniform group. The significant differences ($P \leq 0.05$) were calculated. The statistical analysis was carried out with a Statistica ver. 5.0 software (General Convention and Statistics 1995).

Results and Discussion

Characterisation of Ascorbigen

The ABG synthesised was analysed by ^{1}H NMR and ^{13}C NMR. Chemical shifts were expressed as parts per million (ppm) and were referenced to the residual protons of the solvent. The following shifts were obtained: ^{1}H NMR (300 MHz, CD$_3$OD) δ (ppm): 3.41 (d,1H), 3.61 (d,1H), 3.98 (s,1H), 4.18 (dd,1H), 4.31 (dd,1H), 4.40 (dd,1H), 7.17 (t,1H), 7.23 (t,1H), 7.40 (s,1H), 7.51 (d,1H), 7.81 (d,1H); ^{13}C NMR (50 MHz, CD$_3$OD) δ (ppm): 178.72, 137.52, 129.09, 126.58, 122.24, 119.99, 119.73, 112.04, 108.68, 107.50, 88.29, 80.90, 75.51, 75.49, 31.80. The ^{1}H NMR and ^{13}C NMR spectra of ABG were identical to values reported previously (Preobrazhenskaya et al. 1996). Synthesised ABG was quantified by the HPLC-PAD devise at 280 nm and the purity was superior to 99.5%.

Electrochemical Characterisation of Ascorbigen

The reducing activity of ABG was studied by CV at pH within the range of 3.0–7.0 (Fig. 2). The peak current potential related to the irreversible oxidation of ABG was
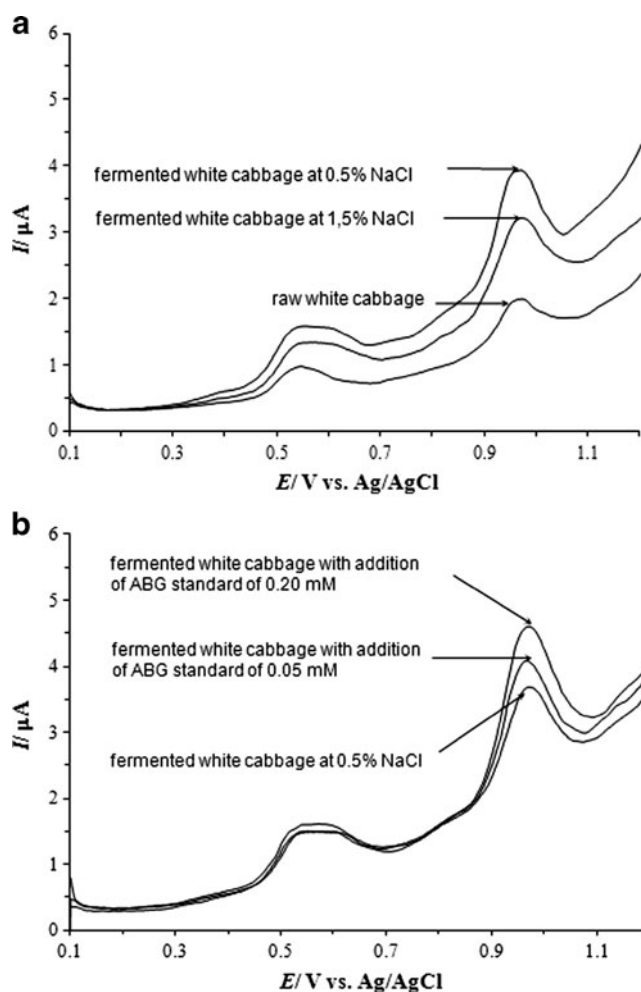

Fig. 5 **a** The DPVs of extracts of raw (*lower line*) and naturally fermented white cabbage at 0.5% (*upper line*) and 1.5% salt level (*middle line*); **b** DPVs of the extract of fermented white cabbage at 0.5% NaCl (*lower spectra*) with the addition of ABG standard at a final concentration of 0.05, 0.1 and 0.2 mM in an extract

Table 2 Content of ABG in raw cabbage (*B. oleracea* L. var. *capitata* cv. Bronco) and 0.5% and 1.5% NaCl sauerkrauts

Material	DPV	HPLC-CoulArray
Raw cabbage	20.5±1.0a	21.6±1.4a
0.5% NaCl sauerkraut	88.1±2.8c	107.3±1.0c
1.5% NaCl sauerkraut	68.4±1.2b	77.4±1.1b

Data are expressed in micromoles per 100 g d.m. and are the means ± standard deviations of three independent determinations. Means within a column followed by different lowercase letters correspond to significant differences ($P \leq 0.05$)
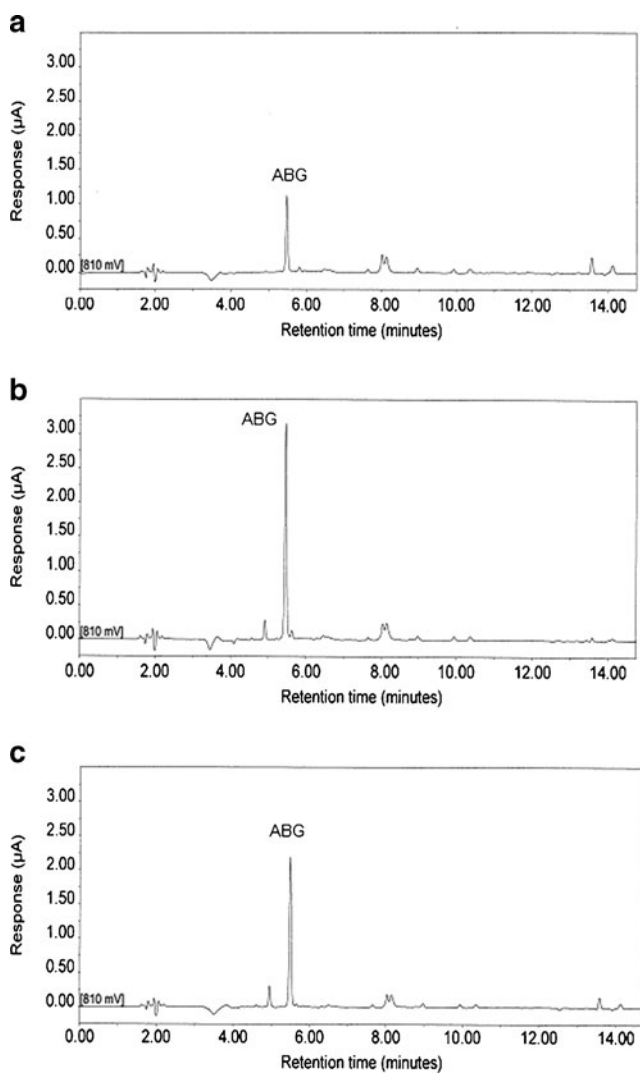

Fig. 6 HPLC-CoulArray chromatograms (applied potential, 810 mV) of extract from **a** raw and naturally fermented white cabbage at **b** 0.5% NaCl and **c** 1.5% NaCl levels. The sample (20 μl) was injected into a Hypersil Gold column (150×4.6 mm, 5 μm, Thermo Scientific) at 30 °C. The chromatogram was developed at a flow rate of 1.2 ml/min in a gradient of mobile phase A (10% acetonitrile, 0.05 M ammonium acetate, pH 5.7) and mobile phase B (80% acetonitrile, 0.05 M ammonium acetate, pH 5.7) as follows: 100–100–50–50–0–100% B at gradient time of 0–1–13–16–21–26 min

shifted towards more positive potentials with decreasing pH values. At pH 4–5.0, which is the pH typical for sauerkrauts, CV studies of ABG at glassy carbon electrode showed oxidation peak centred at $E_p = 0.950$ V and linear response at 0.25 mM was found between pH and anodic peak current potential. Oxidation peak for ascorbic acid by CV was also recorded and occurred at $E_p = 0.376$ V (cyclic voltammogram not shown), excluding any interference between both compounds. The linear response between concentrations of ABG within the range of 0.08–0.75 mM and anodic peak current at selected pH 5.0 was observed (Fig. 3). The DPV offers higher sensitivity when compared to CV method. In this experiment, the linear response of the anodic peak current vs. ABG concentration in a lower range of 0.0125–0.10 mM was provided (Fig. 4) and the oxidation peak related to ABG at pH 5.0 was noted at $E_p = 0.950$ V.

Antioxidant Activity and Reducing Power of Ascorbigen, Raw and Fermented Cabbages

Table 1 collects the antioxidant activity and reducing power of ABG standard measured by PLC and CV methods. The antioxidant activity of ABG against superoxide anion radicals ($O_2^{-\cdot}$) evaluated by PCL was very low (0.0128± 0.0002 mM of Trolox), whilst the reducing power of ABG provided by CV experiments was rather higher (0.847± 0.028 mM of Trolox). Different antioxidant activity of ABG has been found in the literature. Wagner et al. (2008b) indicated that ABG did not scavenge superoxide anion free radicals and showed only little scavenging activity toward 2,2-diphenyl-1-picrylhydrazyl free radicals (DPPH radicals). Similarly, a weaker antioxidant activity of ABG in comparison with ascorbic acid was measured against radicals produced by decomposition of 2,2′-diazobis(2-aminopropane) dihydrochloride (AAPH) has been reported (Joshi et al. 2008). Therefore, antioxidative effects of ABG may also be
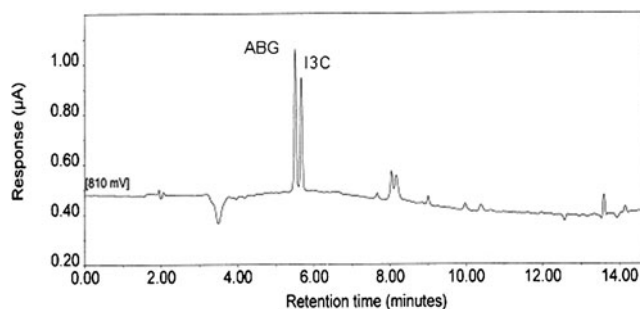

Fig. 7 HPLC-CoulArray chromatogram (applied potential, 810 mV) of the mixture I3C and ABG standard solution at final concentrations of 0.8 and 1.7 μM, respectively. The chromatogram was developed as described in Fig. 6

mediated by other mechanisms than its free radical scavenging per se, as it has been suggested by Wagner et al. (2008b). Furthermore, most of the GLS hydrolysis products do not neutralise free radicals directly but they exert an indirect antioxidant activity, which enhance the activity of xenobiotic-metabolizing enzymes triggering the long-lasting antioxidant activity (Vig et al. 2009).

Antioxidant activity and reducing power of raw and fermented cabbage were evaluated by PCL and CV methods (Table 1) and they were rather larger than those observed for the ABG standard. Among them, 0.5% NaCl sauerkraut showed statistically ($P \le 0.05$) higher antioxidant capacity, followed by the 1.5% NaCl and the raw cabbage. These results indicate that other different compounds present in sauerkrauts than ABG are contributing to the antioxidative and reducing capacities of raw and fermented cabbages measured by PCL and CV methods, respectively.

Application of DPV and HPLC-CoulArray for the Determination of Ascorbigen in Raw and Fermented Cabbages

Since ABG is an electroactive compound, the use of an electrochemical method for its quantitative analyses is feasible. Firstly, differential pulse voltammograms of sauerkraut extracts were recorded at pH 5, which is typical of sauerkraut products. The anodic peak current corresponding to ABG in extracts originated from sauerkrauts was higher than that one related to ABG from raw cabbage extracts (Fig. 5a). The presence of ABG in fermented cabbages was confirmed by the addition of external ABG standard. For this purpose, final concentrations of 0.05 and 0.1 mM ABG standard were added, and the recorded DPVs clearly confirmed the dose-dependent ratio between anodic peak current and ABG concentration (Fig. 5b). Based on the linear response of the anodic peak current vs. ABG standard concentration within a range of 10–100 μM provided by the DPV method (Fig. 4), the content of ABG in raw cabbage and sauerkrauts was calculated (Table 2). The content of ABG in raw and fermented cabbage at 0.5% and 1.5% NaCl was rather similar than what has been previously provided by HPLC-PAD quantification by Peñas et al. (2010) (14, 100 and 75 μmol/100 g d.m., respectively), and differences among methods ranged between 9% and 15%. The results show that DPV represents a simple, fast and low-cost analytical method for screening the ABG content in fermented cabbage products.

To take the most of the electrochemical properties of ABG, the analysis of raw and fermented cabbage was also carried out by the HPLC-CoulArray detector. The analyses were carried out at pH 5.7, as in the case of HPLC-PAD analysis (Peñas et al. 2010). At this pH, the highest sensitivity of the CoulArray detector was achieved at E_p=810 mV and

chromatograms of raw and fermented cabbage at 0.5% and 1.5 % NaCl are shown in Fig. 6. ABG content in raw cabbage and sauerkrauts was obtained in base on the ABG standard curve in a range of 0.82–3.28 μM (correlation coefficients R^2=0.9975) and the results are presented in Table 2. The detection limit was 15 ng/L, with the signal to noise ratio of 3:1. In general, ABG content in raw cabbage was low (~21 to 22 μmol/100 g d.m.) and natural fermentation led to a sharp increment on ABG content to values of 107 and 77 μmol/100 g d.m. for 0.5% and 1.5% NaCl sauerkrauts, respectively. These results are comparable to those previously obtained by HPLC-PDA (100 and 75 μmol/100 g d.m., respectively) (Peñas et al. 2010) and DPV methods (Table 2), and only a 3–15% deviation range between methods was shown. Moreover, analysis of sauerkraut by HPLC-CoulArray showed the absence of I3C; however, this method in one run allows to separate I3C and ABG from solution containing both compounds (Fig. 7). The separation was achieved due to the differences in the retention time, which was 5.551 min for ABG and 5.67 min for I3C. It can be concluded that both electrochemical methods are fully applicable for the screening of ABG amounts in sauerkrauts.

Conclusion

In this study, the spectral behaviour of ABG was applied to develop quantitative analytical methods for the determination of ABG in sauerkraut by DPV and HPLC with CoulArray detection. It was shown that the ABG standard was an electroactive compound, based on its electrochemical behaviour studied by CV and DPV on the GC electrode. ABG showed a significant reducing power provided by CV, whilst its antioxidant activity against $O_2^{-\bullet}$ was negligible. Despite of the common reversed-phase HPLC with UV detection method used for ABG quantification, both electrochemical DPV and HPLC-CoulArray methods have been successfully used for the direct determination of ABG in sauerkrauts.

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