Antioxidant and anticancer activities of *Chenopodium quinoa* leaves extracts – In vitro study

Urszula Gawlik-Dziki\textsuperscript{a,\*}, Michał Świeca\textsuperscript{a}, Maciej Sułkowski\textsuperscript{b}, Dariusz Dziki\textsuperscript{c}, Barbara Baraniak\textsuperscript{a}, Jarosław Czyż\textsuperscript{b}

\textsuperscript{a} Department of Biochemistry and Food Chemistry, University of Life Sciences, Skronna Str. 8, 20-704 Lublin, Poland
\textsuperscript{b} Department of Cell Biology, Jagiellonian University, Gronostajowa Str. 7, 30-387 Cracow, Poland
\textsuperscript{c} Department of Thermal Technology, University of Life Sciences, Doświadczalna Str. 44, 20-280 Lublin, Poland

\textbf{A R T I C L E   I N F O}

Article history:
Received 5 December 2012
Accepted 15 March 2013
Available online 26 March 2013

Keywords:
Chenopodium quinoa
Antioxidant activity
Anticancer activity
Phenolics
Bioaccessibility in vitro

\textbf{A B S T R A C T}

The nutraceutical potential of *Chenopodium quinoa* Leaves (ChL) was assessed through analyses of their phenolic content, elucidation of the effect of ChL phenolic compounds on cancer cell properties and estimation of their antioxidative activity, bioaccessibility and bioavailability in vitro. Considerable amounts of ferulic, sinapinic and gallic acids, kaempferol, isorhamnetin and rutin were observed in the chemical ChL extract and were linked with its inhibitory effect on prostate cancer cell proliferation, motility and cellular competence for gap junctional communication. Both extracts, chemical and obtained after simulated digestion, exerted an inhibitory effect on lipoxygenase activity, paralleled by their considerable chelating, antioxidative, antiradical and reducing power. These observations indicate that phenolic ChL compounds may exert a chemopreventive and anticarcinogenic effect on oxidative stress and ROS-dependent intracellular signaling via synergic effects. The relatively high potential bioaccessibility and bioavailability of the compounds probably responsible for these effects demonstrates the suitability of ChL for dietary supplementation.

\textcopyright 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Phenolic plant compounds have been found to exert diverse biological effects (Fang et al., 2002; Liu, 2004). Nutraceutical properties of dietary plants are commonly considered in terms of their phenolic content. At the epidemiological level, plant polyphenols have been suggested to reduce the risk of cardiovascular diseases, neurodegenerative disorders and diabetes (Arts and Hollman, 2005). Their properties are related to the antibacterial, antiviral, anti-inflammatory and anti-allergenic activities observed in vitro, such as the inhibition of plasma platelet aggregation, cyclooxygenase (COX) activity and histamine release (Cevallos-Casals et al., 2006). Conceivably, the inhibition of inflammatory processes and tumor angiogenesis also participate in the apparent interference of polyphenols with cancer promotion and progression (Dai and Mumper, 2010). However, direct interference of phenolic plant compounds with cancer cell traits crucial for their neoplastic and invasive potential have also been described (Boivin et al., 2009) and, at least in part, ascribed to the antioxidative activity of polyphenols (Gawlik-Dziki et al., 2012).

Actually, most of the antioxidative potential of plant foods, which could be beneficial to human health, is due to the properties of phenolic compounds. Reactive oxygen species (ROS) are produced in all aerobic cells as by-products of oxygen metabolism. When ROS generation overwhelms the cellular antioxidant capacity, oxidative stress ensues. Under these conditions, ROS can oxidize lipids, proteins and nucleic acids, ultimately leading to cell death or transformation. Phenolic compounds can act as reducing agents, free radical scavengers, hydrogen donors and inhibitors of pro-oxidative enzymes (Cai et al., 2004; Gawlik-Dziki et al., 2012a), thus participating in the prevention of DNA adduction formation and enhanced carcinogen elimination. However, they can also exert chemopreventive effects through interference with ROS, which act as secondary messengers in signaling pathways crucial for cancer cell proliferation and invasion (Dai and Mumper, 2010). ROS generation during inflammation is bound, \textit{inter alia}, with lipoxygenase (LOX) activity. Phenolic plant compounds have been suggested as inhibiting inflammation and tumor promotion via deactivation of a range of pro-oxidative enzymes, including inhibition of LOX-mediated arachidonic acid metabolism (Catalano and Procopio, 2005; Liu, 2004; Gawlik-Dziki et al., 2012b). These data justify research on food supplements that would enrich a normal diet with new sources of antioxidative phenolics.

For centuries, *Chenopodium* spp. has been cultivated as a leafy vegetable and subsidiary grain crop in different parts of the world...
(Bhargava et al., 2003). Recently, in a number of countries (especially in Europe) there has been growing interest initiating introduction and research work on Chenopodium quinoa Willd. (Bhargava et al., 2006). The seeds of this pseudocereal have been traditionally consumed as food by the native population of the Andes region (Bhargava et al., 2006). They are rich in proteins and free essential amino acids, starch, minerals and oils, but also contain low amounts of several vitamins and antioxidants (Letelier et al., 2011). On the other hand, little information is available concerning the nutraceutical potential of the green parts of C. quinoa. In particular, pseudocereal leaves, till now treated as worthless waste, are edible and may be consumed in salad, and also used as a valuable supplement for functional food (Gawkli-Dziki et al., 2009). Quinoa leaves contain an ample amount of ash (3.3%), fiber (1.9%), nitrates (0.4%), vitamin E (2.9 mg α-TE/100 g) and Na (289 mg/100 g), vitamin C (1.2–2.3 g/kg) and 27–30 g/kg of proteins (Bhargava et al., 2006). However, little is still known about the phenolic content and the biological activity of phenolic compounds in *C. quinoa* leaves.

The aim of this study was to assess the nutraceutical potential of *C. quinoa* Leaves (CHL) in the context of the bioaccessibility and bioavailability of their phenolic compounds. For this purpose, we analyzed the phenolic content of CHL and estimated its combined bioactivity, using an experimental model based on in vitro cultivated prostate cancer MAT-LuLu and AT-2 cell lines (Gawkli-Dziki et al., 2012a), characterized by different metastatic potentials. The inhibitory effect of phenolic CHL compounds on a set of cellular traits crucial for cancer promotion and progression was observed. The high antioxidative potential of bioaccessible and bioavailable CHL compounds extracted in conditions imitating gastrointestinal processing prompted us to speculate on the high nutraceutical value of CHL.

2. Material and methods

2.1. Chemicals

Ferrozine (3-(2-pyridyl)-5,6-bis-(4-phenyl-sulfonic acid)-1,2,4-triazine), ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)), α-amylase (EC 3.2.1.1), pancreatic, peptic (EC 3.4.23.1), trypsin (EC 3.4.21.4), bile extract, linoleic acid, ammonium thiocyanate and haemoglobin were purchased from Sigma–Aldrich Company (Poznan, Poland). All other chemicals were of analytical grade.

2.2. Plant material

*C. quinoa* seeds var. Faro were purchased from PNOS S.A. in Ożarów Mazowiecki, Poland. Plant material (leaves of *C. quinoa*) was harvested on an experimental farm belonging to the University of Life Sciences in Lublin, Poland. The soil was characterized by mean content of humus, very low phosphorus, potassium and magnesium content, and was acidic in reaction. During vegetation plants were weeded by hand three times, and the inter-rows were cultivated. Leaves were harvested after 90 days of growth and dried at 50°C for 12 h.

2.3. Extraction procedures

For chemical (ethanol; ETOH) extraction 1 g of dry CHL (DW) was homogenized for 1 min using a laboratory blender, extracted in 25 mL ETOH (50%, v/v) for 1 h at room temperature (RT), centrifuged (15 min, 3000g, RT) and then the supernatants were recovered. The procedures were repeated and the supernatants combined. After that, the solvent was evaporated and the remaining water phase was concentrated under reduced pressure. Such processed samples were transferred to columns packed with the stationary phase with chemically bonded octadecyl groups (Sep-Pac C18 columns). Pure ethanol was used to elute the column. For cell proliferation, motility and gap junctional coupling assays (2.4), ethanol was evaporated and the polyphenolic fraction was dissolved in DMSO (1.5 ν/v).

For buffer (PBS) extraction 1 g of dry CHL was homogenized for 1 min using a laboratory blender, extracted in 50 mL PBS (pH 7.4) for 1 h at room temperature (RT), centrifuged (15 min, 3000g, RT) and then the supernatants were recovered. The procedures were repeated and the supernatants combined. For bioaccessibility and bioavailability studies simulated mastication, gastrointestinal digestion and absorption in vitro were performed according to procedures described by Gawkli-Dziki (2012). Simulated saliva solution was prepared by dissolving 2.38 g NaHPO4, 0.19 g KH2PO4, and 8 g NaCl 100 mg of mucin in 1 L of distilled water. The solution was adjusted to pH 6.75 and α-amylase was added to obtain 200 U/mL of enzyme activity. For gastric digestion 300 U/mL of pepsin (from porcine stomach mucosa, pepsin A) in 0.03 mol/L NaCl, pH = 1.2 was prepared. Further, simulated intestinal juice was prepared by dissolving 0.05 g of pancreatin (activity equivalent 4 x USP) and 0.3 g of bile extract in 35 mL 0.1 mol/L NaHCO3. The CHL were subjected to simulated gastrointestinal digestion as follows: 10 g of sample were homogenized in a stomacher laboratory blender for 1 min in the presence of 5 mL of simulated salivary fluid to simulate mastication; and subsequently, the samples were shaken for 10 min at 37°C. The samples were adjusted to pH = 1.2 using 5 mol/L HCl; and subsequently, 15 mL of simulated gastric fluid were added. The samples were shaken for 60 min at 37°C. After digestion with the gastric fluid, the samples were subjected to pH 6 with 0.1 mol/L of NaHCO3 and 15 mL of a mixture of bile extract and pancreatin were added. The samples were adjusted to pH 7 with 1 mol/L NaOH and finally 5 mL of 120 mmol/L NaCl and 5 mL of mmol/L KCl were added to each sample. The prepared samples were subjected to in vitro digestion for 20 min.

Considering that antioxidant absorption proceeds mainly at the intestinal digestion stage, the resulting mixture was transferred to dialysis sacks (D9777-100FT, Sigma–Aldrich), placed in an Erlenmeyer flask containing 50 mL of PBS and incubated in a rotary shaker (2 ± 2 h, 37°C). The PBS buffer, together with the compounds that passed through the membrane (dialysate), was treated as an equivalent of the raw material absorbed in the intestine after digestion.

2.4. Cell proliferation, motility and gap junctional coupling assays

Rat prostate cancer AT-2 and MAT-LyLu cells (the Dunning rat model), HTB-140 and normal mouse 3T3 fibroblasts were cultured as previously described (Miekus et al., 2005). Stock cultures were harvested upon confluence with 0.25% trypsin, and seeded into culture dishes for endpoint experiments. For proliferation assays, CHL extract in DMEM was diluted in culture medium (DMEM supplemented with 10% foetal bovine serum) to obtain final concentrations 1.86, 0.93, and 0.186 mg CHL DW/mL (referring to 10 μM, 5 μM, and 1 μM phenolics calculated as gallic acid equivalents (GAEs), respectively) and administered 24 h after cell seeding (10^5 cells/cm²). The cells were cultivated in the presence of CHL extract for the next 24 h in 6-well dishes (Corning), followed by cell counting with a Burker chamber (Czerneck et al., 2008). For control experiments, culture medium supplemented with 0.25, 0.1% and 0.02% (v/v) DMEM was used. The movement of individual cells was recorded 48 h after cell seeding into culture flasks (25 cm² from Corning; 5 x 10^5 cells/cm²) and 24 h after the administration of the culture medium containing the extract, using a computer-assisted data acquisition system (Leica DM IRE2). The cell trajectories (>50 cells, three independent experiments) were pooled and statistically analyzed (Daniel-Wójcik et al., 2008). The following parameters were estimated: (i) velocity of cell displacement (VCD; μm/h); 4 h; (ii) velocity of cell movement (VCM; μm/h; 4 h) and (iii) cell instantaneous velocity (μm/min). Cx43 expression and gap junctional intercellular coupling (GJIC) was analyzed as described previously (Szpak et al., 2011). Homologous gap junctional coupling was quantified as the percentage of donor cells coupled with at least one recipient cell (coupling index – ccr) and compared with the relevant DMEM control. At the concentration between 0.02% and 0.2% (v/v), DMEM exerted no significant effects on proliferation, motility and GJIC of cultured cells.

2.5. HPLC analysis of phenolic compounds

Samples were analyzed with a Varian ProStar HPLC System separation module (Varian, Palo Alto, CA) equipped with a Varian ChromSpher C18 reverse phase column (25 mm x 4.6 mm) and a Prostar DAD detector, as described by Świeca et al. (2012). The column thermostat was set at 40°C. The mobile phase consisted of 5.4% acetic acid (solvent A) and 50% acetic acid (solvent B), and the flow rate was 1 mL/min. The gradient elution was used as follows: 0 min 100% A, 5 min 100% A, 20 min 90%, 35 min 80%, 50 min 70% A, 60 min 55% A, 70 min 0% A, and 85 min 0% A. At the end of gradient, the column was maintained in solvent A and then in solvent B to equilibrate the column and GJIC after that equilibrated to the initial condition for 10 min. Spectrum analysis and a comparison of their retention times with those of the standard compounds identified the phenolics in a sample. Quantitative determinations were carried out with the external standard calculation using calibration curves of the standards.

2.6. Antioxidant activities

The protective activity of CHL extracts against lipid oxidation was determined as the degree of inhibition of the haemoglobin-catalyzed peroxidation of linoleic acid according to Kuo et al. (1999). Antiradical activity was performed according Re et al. (1999), using an improved ABTS decolorisation assay, whereas chelating potential was determined by the iron(III) reduction method (Gimenez et al., 2001). The effect of CHL extracts on LOX activity was assessed (spectrophotometrically at a temperature of 20°C by measuring the increase in absorbance at 234 nm over a 2 min period) according to Axelroad et al. (1981), while reducing power was elucidated with the approach proposed by Oyaizu (1986). Antioxidant activities (except reducing
power) were determined as EC\textsubscript{50} – extract concentration (mg DW/mL) provided 50% of activity based on a dose-dependent mode of action. Reducing power determined as EC\textsubscript{50} is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis.

2.7. Theoretical approach

For a clear illustration of the relationships between the activity of extracts, and bioaccessibility and bioavailability of their phenolic compounds, the following parameters were determined (Gawlik-Dziki, 2012):

- the antioxidant bioaccessibility index (BAC), which is an indication of the bioaccessibility of antioxidative compounds
  
  \[
  \text{BAC} = A_l/A_o
  \]

- the antioxidant bioavailability index (BAV):
  
  \[
  \text{BAV} = A_l/A_r
  \]

- the antioxidant bioefficiency index (BEF), which is an indication of the bioactivity of bioavailable antioxidative compounds:
  
  \[
  \text{BEF} = A_l/A_o
  \]

where \( A_l = \text{EC}_{50} \) of raw extract (after PBS extraction); \( A_o = \text{EC}_{50} \) of extract after simulated gastrointestinal digestion, and \( A_r = \text{EC}_{50} \) of extract after simulated intestinal absorption.

2.8. Statistical analysis

Unless stated otherwise, the experimental results were mean ± SD of three parallel experiments (N = 9). The obtained data were subjected to a statistical analysis and the consequent evaluations were analyzed using a variance analysis. The statistical differences in 2.4 were estimated with the paired t-Student (proliferation) and non-parametric Mann–Whitney tests (motility), and in 2.6, through Tukey’s test. Statistical tests were evaluated by using Statistica 6.0 software (StatSoft, Inc., Tulsa, USA). All the statistical tests were carried out at a significance level of \( \alpha = 0.05 \).

3. Results and discussion

3.1. Identification of CHL phenolic compounds

Chenopodium spp. has long been considered a potential dietary supplement rich in bioactive phenolic compounds. For instance, C. pallida\textsubscript{cula} (\textit{canihua}) contains considerable amounts of vanillic acid, whereas quercetin was found in 7, kaempferol in 5, isorhamnetin in 2, and herbacetin in one Chenopodium species. Importantly, kaempferol was encountered in the aerial parts of \textit{C. album} and \textit{C. murale} as well as in \textit{C. pallida\textsubscript{cula}}. Similarly, cinnamic, sinapinic, ferulic, vanillic, caffeic acid (and their derivatives) have been isolated from the leaves of \textit{C. album}. The occurrence of quercetin has been reported in the aerial parts and fruits of \textit{C. ambrosioides} and in the aerial parts of \textit{C. album}, \textit{C. botrys}, \textit{C. hybrida\textsubscript{m}}, \textit{C. murale}, \textit{C. pallida\textsubscript{cula}} (Kokanova-Nedialkova et al., 2009; Laghari et al., 2011). Identification of quercetin in CHL, paralleled by our previous studies (Gawlik-Dziki et al., 2012c), which demonstrated a relatively high phenolic content in CHL, prompted us to assess the amounts of major phenolic acids and flavonoids in CHL.

HPLC analyses allowed for the identification of the aglycones of ten major phenolic acids and four flavonoids in the polyphenolic fraction of chemical (ethanolic) CHL extract. The main phenolic acids were ferulic, sinapinic and gallic acids, whereas kaempferol and isorhamnetin were the most abundant flavonoids (Table 1). It should also be emphasized that CHL contained large amounts of rutin. Previously, vanillic acid glucosyl ester had been found in \textit{C. quinoa} seeds (Kokanova-Nedialkova et al., 2009); however, no comprehensive data concerning the phenolic composition of CHL are available. Our study fills this gap and shows that CHL can potentially serve as a rich source of phenolic compounds, characterized by a wide-range spectrum of biological activities, including antioxidative potential and cytostatic effects. Because the high nutraceutical potential of these compounds has been suggested (Gawlik-Dziki et al., 2012b), our observations suggest CHL could be introduced into the standard diet as a nutraceutically valuable green leafy vegetable.

3.2. Cytostatic and anti-invasive effects of chemical CHL extract in vitro

We have previously demonstrated the suitability of the approach based on the analyses of rat prostate cancer cell proliferation, motility and gap junctional coupling for the assessment of the bioactivity of phenolic plant compounds (Gawlik-Dziki et al., 2012b). Therefore, we further used this model to estimate the range of concentrations of chemically extractable CHL polyphenols effectively interfering with these cell properties. Polyphenolic CHL fraction dissolved in DMSO evoked a significant and dose-dependent cytostatic effect in both cell lines when administered at concentrations between 0.186 and 1.86 mg CHL DW/mL (i.e. 1–10 μM GAE). AT-2 cells were more sensitive to the extract administered at lower concentrations; in contrast, a more pronounced effect was observed in MAT-LyLu populations in the presence of higher concentrations of CHL extract (0.93 mg CHL DW/mL; 5 μM GAE). A complete proliferation block was observed in both populations in the presence of CHL upon administration at 1.86 mg DW/mL (Fig. 1A). Administration of the extracts from \textit{C. quinoa} seeds and sprouts exerted only minute effects on the proliferation of both prostate cancer cell lines (data not shown). This demonstrates that the cytostatic effect of CHL extract results from its unique composition.

Previously, we reported corresponding differences in the reactivity of these cells to phenolic compounds of broccoli extracts (Gawlik-Dziki et al., 2012b). Current data indicate that the application of phenolic compounds of CHL may also be considered in terms of prostate cancer chemoprevention, whereas their wide-spectrum of activity is illustrated by their cytostatic effect on melanoma HTB-130 cells (Fig. 1A). Notably, lower CHL extract concentrations exerted no significant cytostatic effect on proliferative activity of normal 3T3 cells. The effect of CHL extract at 1.86 mg DW/mL on their growth is not surprising, because similar regulatory pathways determine the proliferation of normal and cancer cells. However, their lower sensitivity to the extract may substantiate the notion about the nutraceutical potential of CHL compounds.

Importantly, time-lapse analyses also revealed an inhibitory effect of CHL extracts (0.93 mg CHL DW/mL; 5 μM GAE) on the motility of MAT-LyLu cells as illustrated on circular diagrams depicting the trajectories of control and CHL extract-treated cells (Fig. 1B). Analyses of cell instantaneous velocities revealed a gradual inhibition of cell motility during the experiment. aberrant cell

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>μg/g DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>162.85 ± 12.51</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>10.28 ± 0.68</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>37.55 ± 2.15</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>22.67 ± 1.65</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>18.69 ± 0.98</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>33.31 ± 2.65</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>762.29 ± 42.31</td>
</tr>
<tr>
<td>Sinapinic acid</td>
<td>193.48 ± 11.34</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>1.49 ± 0.25</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>2.29 ± 0.21</td>
</tr>
<tr>
<td>Kemperferol</td>
<td>46.00 ± 5.21</td>
</tr>
<tr>
<td>Quercetin</td>
<td>6.88 ± 0.58</td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>3.06 ± 0.24</td>
</tr>
<tr>
<td>Rutin</td>
<td>62.12 ± 5.65</td>
</tr>
</tbody>
</table>
proliferation is crucial for prostate cancer promotion (Wang and Tindall, 2011), while the formation of actively migrating cancer cell subpopulations is a key step in cancer progression (Friedl et al., 2004). Therefore, the inhibitory effect of ChL extract on both parameters indicates that ChL may serve as a valuable supplement for the combination therapy of prostate cancer. Furthermore, direct gap junction-mediated intercellular exchange of small metabolites is supposed to facilitate cancer invasion (Czyz et al., 2012). Thus, the inhibition of Cx43 expression, which results in attenuation of dye transfer from donor to recipient cells observed in extract-treated MAT-LyLu populations (C), dose-dependence of this effect in MAT-LyLu and AT-2 populations is depicted in (D). Proliferation (A) and GJIC (D) of ChL extract–treated cells were plotted as % of relevant DMSO control. Bars represent means ± SEM. *p < 0.05 as determined by the paired t-Student test obtained from three independent experiments (N = 3). Trajectories of control (0.1% DMSO) and ChL extract–treated MAT-LyLu cells (B) are presented in the form of a circular diagrams (axis scale in μm) drawn with the initial point of each trajectory placed at the origin of the plot. †p < 0.001 determined with the Mann–Whitney test (N = 3). Plot in (B) depicts the instantaneous velocities of MAT-LyLu cells in the presence of ChL extract during a time-lapse experiment against averaged DMSO control value (dotted line). Bar = 25 μm.

3.3. Antioxidant potential of chemical ChL extract

In several reports, the antioxidant activity of the extracts from Chenopodium spp. has been described. For instance, the essential oil from C. ambrosioides exhibited a high ability to quench ABTS radicals (Kokanova-Nedialkova et al., 2009). Therefore, the suitability of C. ambrosioides extracts (alone and in combination with lecithin and citric acid) for stabilization of unsaturated compounds in the food and pharmaceutical industry has been suggested. The antioxidant capacity of the water-soluble and water-insoluble compounds of C. pallidicaule has been revealed by FRAP and ABTS methods and, at least in the case of the water-soluble extract, attributed to resorcinol activity. Flavonoids, isolated from C. quinoa seeds, exhibited antioxidant activity in DPPH tests. However, nothing is known about the antioxidant activity of phenolic ChL compounds. Previously published data have indicated that the antioxidant activities of phenolic plant compounds are correlated with anticancer and anti-atherosclerotic potential (Gawlik-Dziki et al., 2012b; Hseu et al., 2008). Therefore, we further assayed the antioxidant potential of ethanolic ChL extract.

Phenolic ChL compounds were able to prevent lipid oxidation, while their reducing power and antiradical activity were significantly lower (Fig. 2). Additionally, relatively low EC₅₀ values (i.e.
the concentration needed to cause 50% of the antioxidant effect, meaning that lower EC50 relate to higher antioxidant activity) illustrate that phenolic ChL compounds act as effective LOX inhibitors, perhaps due to their metal chelating abilities (Fig. 2). These data may confirm that the additive or synergic action of several chemical compounds, which act at single or multiple target sites, accounts for the observed reactions of cancer cells to ChL. While a number of signaling pathways crucial for cancer cell proliferation and motility can be affected by polyphenols (Liu, 2004), our previous observations revealed that AT-2 and MAT-LyLu cells were sensitive to the antioxidative activities of broccoli sprout extracts (Gawlik-Dziki et al., 2012b). LOX activity is crucial for prostate inflammation and cancer development (Catalano and Procopio, 2005; Goodman et al., 2011; Liu, 2004), therefore future studies on the mechanisms of the effects of ChL compounds on cancer cells should be focused on this enzyme. However, the possibility of ROS scavenging-independent action of identified and/or still unknown compounds should also be taken into account in data interpretation (Liu, 2004; Dashwood, 2007). On the other hand, the unique antioxidant action of ChL extracts – LOX inhibition and strong chelating power (protection against Fenton reaction) – may also suggest their anti-atherosclerotic potential (Hseu et al., 2008). For instance, their high ability to protect lipids against oxidation may interfere with the oxidative damage of erythrocyte membranes, which are sensitive to this process because of their high polyunsaturated fatty acid content. The pro-oxidative potential of erythrocytes, resulting from their high cellular oxygen and haemoglobin concentrations, along with erythrocyte lipid peroxidation has been implicated in normal cell aging, and associated with a variety of pathological events (Hseu et al., 2008). It is also supposed that increased intake of the antioxidants is associated with decreased incidence of new or recurrent cardiovascular disease via the inhibition of LDL oxidation (Klevinld et al., 1994; Stephens et al., 1996).

3.4. Determination of bioaccessibility and bioavailability of ChL antioxidants in vitro

The bioaccessibility and bioavailability of dietary polyphenols are principally relevant to their beneficial effects. Bioaccessibility is defined as the amount of a compound released from the solid food matrix, thereby capable of passing through the intestinal barrier. However, the proportion of the compound that is digested, absorbed, and metabolized through the normal pathways, referred to as its bioavailability, is far more important for the nutraceutical potential of the food (D’Archivio et al., 2010). The bioavailability of each polyphenol is different, and there is no correlation between the quantity of polyphenols in food and their bioavailability in the human body.

In order to determine the potential bioaccessibility and bioavailability of the ChL compounds bearing antioxidative potential, we further analyzed the antioxidative properties of raw ChL extracts (PBS) and those extracts subjected to simulated gastrointestinal processing (GD), and absorption (GDA). As shown in Fig. 3, the release of the compounds acting as effective metal ions chelators, inhibitors of lipid peroxidation, reducing agents and free radical scavengers could be observed during PBS extraction. A significant elevation of these activities in GD extracts (as compared to the PBS extracts) was observed, which may indicate the high bioaccessibility of bioactive ChL compounds (and/or potential protective effects for the upper gastrointestinal tract). A significant decrease in the LOX inhibiting activity of GD extract was observed; however, this effect was reversed upon the absorption procedure. In general, the high bioavailability of ChL compounds was illustrated by the analyses of GDA extracts, which indicated that all the tested antioxidants, except reductive compounds, easily permeated a dialysis membrane. In similar, BAC values (Fig. 4) demonstrated that ChL contained highly bioaccessible antiradical and reductive compounds, whereas the highest bioavailability was found for inhibitors of lipoxygenase and lipid oxidation. Taking into account the bioefficiency factor (BEF) values, it should be concluded that quinoa leaves are a good source of bioavailable, antioxidative compounds, which act in a multifaceted manner.

Notably, GDA extract activities were similar to those observed for chemical (ethanolic) ChL extract. This indicates that phenolic ChL compounds might account for the bulk of the antioxidative potential of ChL and/or that the lower bioavailability of some polyphenols was compensated by other classes of bioactive and bioavailable substances. In contrast to synthetic pharmaceuticals based upon single chemicals, many phytomedicines exert their beneficial effects through synergistic interactions. They are of vital importance for explaining difficulties in isolating a single active ingredient and they explain the efficacy of apparently low doses of active constituents in plant-derived food, e.g. vegetables (Kira-kosyan et al., 2010). This idea has been adopted by pharmacologists when exploring combinations of several metabolites in multi-target therapy. In the light of previously published data concerning phenolic antioxidant bioavailability, elevation of the
studied activities in GDA extracts may also be caused by interaction between constituents present in the fluid after in vitro absorption (Gawlik-Dziki, 2012).

It must, however, be noted that both native food compounds and their metabolites may play a role in the prevention of ROS-related diseases. In general, only aglycones can be absorbed in the small intestine. However, most polyphenols are present in food in the form of esters, glycosides, or polymers that cannot be absorbed in native form (D’Archivio et al., 2007). Prior to absorption, these compounds must be hydrolyzed by intestinal enzymes or by bacterial degradation in the large intestine. As a consequence of the extensive modification that occurs in intestinal and liver cells, their forms reaching the blood and tissues are different from those present in food. Thus, it is difficult to identify and to evaluate the biological activity of all their physiological metabolites in vitro (Pandey and Rizvi, 2009). The experimental model used in this study has been developed as a simple, cheap, and reproducible approach for investigating the bioactivity of food components in the context of their bioaccessibility and bioavailability (Manach et al., 2005). It cannot fully cover the specific role of digestive stability, release from the food matrix, and the efficiency of transepithelial transport, strongly dependent on the hydrophobicity of the compounds. However, our previous studies, showing that about 80% of total ChL phenolics are bioavailable in vitro (Gawlik-Dziki et al., 2012c) and our current data, confirm the high nutraceutical potential of the green parts of C. quinoa.

4. Conclusion

A high content of polyphenols with well documented antioxidant activity, which includes free radical scavenging, reducing power, metal chelation, lipid protection against oxidation and LOX inhibition, confirms the strong nutraceutical potential of ChL, relevant not only to cancer development, but also to inflammatory processes. To date no data have been published on the bioaccessibility and bioavailability of potentially bioactive ChL phytochemicals. Our observations concerning the chemical composition and bioactivity in vitro of ChL fill this gap and open new perspectives for the introduction of ChL into the normal diet, at least as an addition. LWT – Food Sci. Technol. 42, 137–143

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This scientific study was financed by the Polish Ministry of Scientific Research and Higher Education (Grant NN312233738) and the Polish National Science Centre (Grant 2011/01/B/NZ3/00004).

References


