Research Article
The Potential Health Benefits of Polyphenol-Rich Extracts from *Cichorium intybus* L. Studied on Caco-2 Cells Model

Elena Azzini, Giuseppe Maiani, Ivana Garaguso, Angela Polito, Maria S. Foddai, Eugenia Venneria, Alessandra Durazzo, Federica Intorre, Lara Palomba, Maria L. Rauseo, Ginevra Lombardi-Boccia, and Fabio Nobili

Council for Agricultural Research and Economics (CREA), Research Center for Food and Nutrition, Via Ardeatina 546, 00178 Rome, Italy

Correspondence should be addressed to Elena Azzini; elena.azzini@entecra.it

Received 7 September 2015; Revised 28 October 2015; Accepted 28 October 2015

Academic Editor: Gregor Drummen

Copyright © 2016 Elena Azzini et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Phytochemicals can exert their bioactivity without reaching the systemic circulation; scarcely absorbed antioxidants might reach the large bowel contributing to protection from oxidative damage-induced gastrointestinal diseases. In the present work, we aimed to study the relationship between potential activity of polyphenol-rich extracts from *Cichorium intybus* L. and changes in morphological characteristics on Caco-2 cells. Phytochemicals content (carotenoids and flavonoids) and total antioxidant activity of Red Chicory of Treviso and Variegated Chicory of Castelfranco were evaluated. The bioactivity of polyphenol-rich extracts from chicories was studied in *in vitro* Caco-2 cell monolayers model. Morphological characteristics changes to test the antioxidant and/or prooxidant effect were verified by histological analysis and observed by Electronic Scansion Microscopy (SEM). On Caco-2 cell model, the polyphenols fractions from chicories have indicated a moderate antioxidant behavior until 17 \( \mu \)M concentration, while 70 \( \mu \)M and 34 \( \mu \)M exert cytotoxic effects for Treviso’s and Castelfranco’s Chicory, respectively, highlighted by TEER decreasing, increased permeability, and alteration of epithelium. Our findings support the beneficial effects of these products in counteracting the oxidative stress and cellular damage, induced *in vitro* on Caco-2 cell model, through interaction with the mucopolysaccharide complexes in the glycocalyx, maintaining *in vivo* a healthy and effective intestinal barrier.

1. Introduction

Chicory, a plant genus typical of Mediterranean area, is native to Europe, Western Asia, and North America and its colour varies from white to red [1]. “Red Chicory of Treviso” and “Variegated Chicory of Castelfranco,” with PGI according to EU rules, are strongly linked to their territory and grown according to traditional cultivation techniques. These products have acquired great interest for their organoleptic and nutritional characteristics. As well known, the consumption of phytochemicals from fruits and vegetables can improve the prevention of several chronic degenerative pathologies [2, 3]. Phytochemicals content may be affected by several factors: genetic characteristic, environmental aspects, agronomic practices, and postharvest conditions [4, 5]. Genetic factors exert great influence on nutritional and phytochemicals content, between and within vegetables species. Climate condition, light exposure, temperature, relative humidity, and luminous intensity are specific parameters that affect food quality. In particular, the choice of an appropriate agronomic practice could improve the levels and the profile of bioactive compounds. Among the vegetal crops, red chicories are attractive because they may be consumed either raw or cooked. In particular, they are commonly eaten raw in salad during winter months when most vegetables are not in season. In fact, red chicories are particularly resistant to low temperatures [6] and their availability throughout the year is an important source of micronutrients during the coldest season. The red color is caused in large part by the presence of water-soluble pigments, anthocyanins, but several works show that the red-leaved varieties of *Cichorium intybus* L. have the highest content of polyphenols among the leafy vegetables that are consumed raw [7, 8]. Changes in phytochemicals content in agricultural production take on
a particular importance in our diet. The bioactive compounds in foods, such as vitamins, carotenoids, and polyphenols, seem to be able to modulate one or more metabolic processes, which result in the promotion of better health [9]. The most accepted explanation for the protective effect of food probably derives from the observation that different plant phytochemicals may act as “scavengers” of free radicals, “quenchers” singlet oxygen, or metal chelators [10, 11] and therefore induce protection against oxidative damage through antibacterial, anti-inflammatory, hepatoprotective, anticarcinogenic, and vasodilator actions.

Recently, D’Evoli et al. [12] have shown that the high levels of antioxidant anthocyanins present in Red Chicory exert a direct scavenging effect against ROS formation in terms of antioxidant and cytoprotective activities as well as antiproliferative activity in Caco-2 cell. In the present work, we aimed to study the relationship between potential activity of polyphenol-rich extracts from chicheries and morphological and chemical/physical changes in Caco-2 cellular line. To this purpose, bioactive compounds content (carotenoids and flavonoids) and total antioxidant activity were evaluated in Early and Late Red Chicory of Treviso and Variegated Chicory of Castelfranco. In addition, the bioactivity of polyphenol-rich extracts from chicheries in in vitro Caco-2 cell monolayer model was studied. Morphological characteristics changes to test the antioxidant and/or prooxidant effect were verified by histological analysis and observed by Electronic Scansion Microscopy (SEM).

2. Materials and Methods

2.1. Reagents. All solvents were purchased from Carlo Erba (Milan, Italy), BDH (Poole, England), and Merck (Darmstadt, Germany). 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) was from Fluka (Switzerland). Phosphate-buffered saline (PBS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and ascorbic acid were provided by Sigma-Aldrich Srl (Milan, Italy). AAPH (2,2′-azobis (2-amidinopropane) dihydrochloride) (WACO Chem., Richmond, VA, USA) was used as a source of hydrophilic radicals. Double distilled water (Millipore, Milan, Italy) was used throughout the study.

2.2. Cichorium intybus L. Red Chichories of Treviso (two varieties: Early and Late) samples were grown in the region including Quinto (Treviso), Zero Branco (Treviso), and Scorzé (Venezia), while PGI samples of Variegated Chicory of Castelfranco come from Due Carrare (Padova), Mira (Venezia), and Monselice (Padova). A total weight of 5 kg of each variety for each locality was collected at harvesting time and delivered to the laboratory. Only the edible portion of the samples was utilized for analysis, according to the following methods.

2.3. Analytical Methods. The extraction of some flavonoids (quercetin, kaempferol, and apigenin) was performed as described by Hertog et al. [13] and the quantitative analysis through a system-ESA HPLC with electrochemical detector as reported by Azzini et al. [14]. Carotenoids were extracted from Treviso’s and Castelfranco’s chicory according to the method described by Sharpless et al. [15]. For the quantification, the samples were analyzed by high pressure liquid chromatography (HPLC) as reported by Maiani et al. [16]. Total Antioxidant Capacity (TAC) was evaluated using two different assays: Ferric Reducing Antioxidant Power (FRAP) [17] and Trolox Equivalent Antioxidant Capacity (TEAC) method [18, 19]. The results of each analysis have been performed in triplicate.

2.4. Transepithelial Electrical Resistance (TEER) Evaluation. For TEER evaluation, the polyphenol fractions from Red Chicory of Treviso and Variegated Chicory of Castelfranco hydrolysed extracts have been studied on Caco-2 cell monolayers to test the changes in tight-junction (TJ) permeability by TEER, the phenol red passage, and histological analysis.

The cellular line was treated with increasing concentration of two polyphenols extracts (0.2, 1.3, 10, 17, 34, and 70 μM), for 180 minutes, to simulate in vivo physiological processes. The variations of transepithelial potentials and phenol red permeability were recorded at time intervals (30’). In the experiment, the cells were seeded onto polycarbonate filter cell culture chamber inserts (diameter 6.5 mm, area 0.33 cm², and pore diameter 0.4 μm) at density of 1.5 × 10⁵ cells per filter and placed in a multiwell Falcon. The filter divided the chamber into two parts, apical and basal, that represent the lumen and the basal area of the gastroenteric system. Into two chambers, TEER measurement for assessment of tight-junction permeability was performed using the Millicell ERS apparatus (Millipore, Bedford, MA, USA) according to the manufacturer’s instruction. After calibrating the resistance system, the electrical resistance of the monolayer was measured by placing one electrode on either side of the polycarbonate filter [20, 21]. The results of each analysis have been performed in triplicate and expressed as ΩH M × cm⁻².

Cells reached confluence and differentiation within 15–20 days. During this time, cellular morphology was monitored and checked with a Leitz Diavert inverted light microscope.

2.5. Histological Analysis. The cellular monolayer was isolated together with the filter and fixed in Bouin (an aqueous solution composed of picric acid, acetic acid, and formaldehyde) for about 12 hours, then dehydrated using the alcohol ascending scale (70%, 80%, 90%, 95%, and 100%), and finally enclosed into resin blocks of polymerized epoxy (GMA, J134K polyscience Inc., Warrington, PA, USA). Then, the samples were glued on embedded stubs and cut into 3 μm sections with a Micron rotary microtome (Zeiss Germany) appropriately assembled so as to use a crystal blade according to Ralph’s modification [22, 23]. The sections glued to the microscope slides were stained with Harris’ hematoxylin and eosin [24, 25]. The preparations were made permanent with slide covers and sealed with resin (Eukitt, mounting medium, BDA Laboratories Supplies Pool, England). All sections (3 μm) were examined for histological changes by Diaplan 22 light microscopy (Leitz Germany) as shown in Figure 1. All
3. Results

3.1. Phytochemical Characterization. According to several authors, flavonoid components could be used as potential markers for the analysis of herbs and plants for human consumption [26–28].

With regard to the qualitative analysis of flavonoids (free plus conjugated forms), Figure 1 displays representative HPLC-DAD gradient array chromatograms, while their identification is reported in Table 1. The varied flavonoids content amongst different varieties and growing locations is summarized in Table 2. Comparing the growing locations, there were no significant differences in the flavonoids content, while significant differences were recorded between varieties. Quercetin was found as the most abundant flavonoid in Red Radish of Treviso varieties (90.04 ± 25.16 mg/kg and 97.88 ± 33.94 mg/kg, resp., in Late and Early Red Chicory) with respect to the Variegated Chicory of Castelfranco (14.20 ± 4.51 mg/kg).

The kaempferol was significantly different among cultivars and varieties. Its content in the Late Red Chicory of Treviso (22.80 ± 5.84 mg/kg) variety was significantly higher (P < 0.05) comparing to Early Red Chicory of Treviso (12.35 ± 7.65 mg/kg) and Variegated Chicory of Castelfranco (11.80 ± 3.64 mg/kg).

There were no significant differences in the apigenin content between cultivar and production area on average range from 2.60 ± 1.40 to 3.58 ± 0.29 mg/kg, respectively, for Early Red Chicory of Treviso and Variegated Chicory of Castelfranco.

Our data (Table 3) indicate that lutein and β-carotene were the main carotenoids (ranging from 1.19 ± 0.24 to 2.40 ± 0.61 mg/kg and from 0.19 ± 0.02 to 0.48 ± 0.15 mg/kg, resp.). A significant difference (P < 0.05) was present between β-carotene content of Late Red Chicory of Treviso (0.38 ± 0.08 mg/kg) and Early Red Chicory of Treviso (0.22 ± 0.04 mg/kg). Variegated Chicory of Castelfranco showed a mean of β-carotene content equal to 0.35 ± 0.14 mg/kg. In the Early Red Chicory of Treviso, the mean lutein content was 2.16 ± 0.28 mg/kg with significantly higher levels (P < 0.05) than Late Red Chicory of Treviso (1.27 ± 0.20 mg/100 g) and Variegated Chicory of Castelfranco (1.20 ± 0.27 mg/100 g).

After evaluating the content of individual antioxidants, the cooperative action of bioactive molecules in the different specimens of Cichorium intybus L. was evaluated by total antioxidant capacity (TAC). Figure 2 shows the synergistic effects of various antioxidants measured by FRAP (mmol/kg) and TEAC (mmol trolox/kg) methods.
Table 2: Flavonoids content amongst different varieties by growing locations (mg/kg).

|                  | Quercetin (mg/kg) | Kaempferol (mg/kg) | Apigenin (mg/kg) |
|------------------|-------------------|-------------------|------------------|
| **Late Red Chicory of Treviso** |                   |                   |                  |
| Zero Branco      | 83.92 ± 15.08     | 21.43 ± 2.19      | 2.10 ± 0.61      |
| Scorzè           | 95.89 ± 26.51     | 26.23 ± 10.48     | 3.24 ± 0.82      |
| Quinto           | 90.31 ± 33.9      | 20.94 ± 4.86      | 3.05 ± 1.12      |
|                  | 90.04 ± 25.16     | 22.80 ± 5.84      | 2.80 ± 0.85      |
| **Early Red Chicory of Treviso** |                   |                   |                  |
| Zero Branco      | 101.50 ± 40.20    | 14.42 ± 8.40      | 3.98 ± 1.81      |
| Scorzè           | 100.23 ± 42.4     | 14.51 ± 9.80      | 1.51 ± 0.80      |
| Quinto           | 91.89 ± 29.22     | 8.11 ± 5.38       | 3.62 ± 0.05      |
|                  | 97.88 ± 33.94     | 12.35 ± 7.65      | 3.58 ± 0.29      |
| **Variegated Chicory of Castelfranco** |                   |                   |                  |
| Monselice        | 15.32 ± 3.54      | 8.68 ± 2.16       | 3.33 ± 0.44      |
| Mira             | 14.47 ± 4.92      | 13.5 ± 3.81       | 3.78 ± 0.38      |
| Due Carrare      | 12.8 ± 5.08       | 13.21 ± 4.97      | 3.62 ± 0.05      |
|                  | 14.20 ± 4.51      | 11.80 ± 3.64      | 3.58 ± 0.29      |

ANOVA: $P < 0.05$ a versus b by column.

Table 3: Carotenoids (lutein and $\beta$-carotene) means values (mg/kg) by different cultivars of *Cichorium intybus* L. and by production area.

|                  | Lutein (mg/kg) | $\beta$-carotene (mg/kg) |
|------------------|----------------|--------------------------|
| **Late Red Chicory of Treviso** |               |                          |
| Zero Branco      | 1.27 ± 0.17    | 0.27 ± 0.06              |
| Scorzè           | 1.19 ± 0.24    | 0.20 ± 0.01              |
| Quinto           | 1.41 ± 0.07    | 0.19 ± 0.02              |
|                  | 1.27 ± 0.20    | 0.22 ± 0.04              |
| **Early Red Chicory of Treviso** |               |                          |
| Zero Branco      | 2.12 ± 0.89    | 0.45 ± 0.02              |
| Scorzè           | 1.97 ± 0.11    | 0.35 ± 0.01              |
| Quinto           | 2.40 ± 0.61    | 0.34 ± 0.09              |
|                  | 2.16 ± 0.28    | 0.38 ± 0.08              |
| **Variegated Chicory of Castelfranco** |             |                          |
| Monselice        | 1.28 ± 0.13    | 0.48 ± 0.15              |
| Mira             | 1.26 ± 0.36    | 0.35 ± 0.11              |
| Due Carrare      | 1.07 ± 0.4     | 0.23 ± 0.01              |
|                  | 1.20 ± 0.27    | 0.35 ± 0.14              |

ANOVA: $P < 0.05$ a versus b by column.

Our results highlighted the highest FRAP values of Late Red and Early Red Chicories of Treviso with respect to Variegated Chicory of Castelfranco (11.70 ± 1.92 mmol/kg and 9.93 ± 3.11 mmol/kg versus 8.76 ± 4.46 mmol/kg, resp.). No differences by varieties and production area were present in FRAP (mmol trolox/kg) results.

Significant differences were present in TEAC (mmol trolox/kg) levels. TEAC values of Late Red Chicory of Treviso (4.54 ± 0.88 mmol trolox/kg) and Early Red Chicory of Treviso (5.32 ± 1.76 mmol trolox/kg) were higher ($P < 0.05$) compared with Variegated Chicory of Castelfranco (2.12 ± 0.74 mmol trolox/kg).

3.2. Cell-Based Assays. Differently from other food components, phytochemicals can exert their bioactivity without reaching the systemic circulation. Scarcely absorbed antioxidants might reach the large bowel contributing to protection from oxidative damage-induced gastrointestinal diseases [29]. There are several reports about pharmacological actions and anti-inflammatory effects of chicory [30, 31].

TEER measurements are routinely used to characterize monolayer integrity in the context of cell monolayer permeability experiments, or to quantify permeability changes, for example, as a consequence of paracellular permeability enhancers [32, 33].
Utilizing polyphenol fractions from Red Treviso’s and Castelfranco’s Chicory, the changes in TJ permeability were tested on Caco-2 cell line in monolayers culture. TEER measurements at increasing phenolic concentrations are shown in Figures 3(a) and 3(b), respectively, for Treviso Red Chicory and Variegated Chicory of Castelfranco.

Upon treatment with Red Chicory of Treviso’s and Variegated Chicory of Castelfranco’s polyphenolics extract, as indicated by TEER values, the results showed a monolayer equilibrium model (healthy monolayer cells) at 0.2-1.3-10-17 μM extract concentration. Until 17 μM polyphenolic extracts concentration, the TEER measurements hold out a plateau, indicating the tightness of TJs and the absence of direct interaction between epithelial Caco-2 cells and chicory extracts as confirmed by histological analyses. Figures 4(a) and 4(b) display the 17 μM effect upon both treatments. The ultrastructural cytological analysis by SEM highlighted and confirmed the absence of morphological change or extracts activity on the cellular monolayer (Figures 5(a) and 5(b)).

The concentrations of 70 μM and 34 μM showed a high toxicity, respectively, for Treviso’s and Castelfranco’s Chicory extracts tested. These treatments produce lowering of TEER values (Figures 3(a) and 3(b)), highlighted by increased permeability of TJs (phenol red) and by alteration of epithelium. These concentrations promote cellular necrosis in Caco-2 cells monolayer as shown in histological analysis and confirmed by SEM observations (Figures 6(a), 6(b) and 7(a), 7(b) for Treviso’s and Castelfranco’s Chicory, resp.).

To better understand their bioactivity and attempting to demonstrate the probable prebiotic role of these extracts, Caco-2 oxidative stress was induced by adding 2,2’ azobis (2-amidinopropane) dihydrochloride (AAPH) to the cell culture medium. The interactions between polyphenol-rich Red Chicory of Treviso extracts against AAPH-induced oxidative stress are reported in Figure 8(a). The progressive TEER increases indicated that, upon 0.2, 1.3, and 10 μM, chicory extracts treatment displayed a strong antioxidant activity that appeared to be able to counteract the peroxidative trigger.
induced by AAPH, suggesting a cellular membrane integrity and confluence restoring. Only 0.2 μM Variegated Chicory of Castelfranco extract exhibited a low antioxidant effect (Figure 8(b)) that promotes a cell damage recovery resulting by slight TEER increase.

4. Discussion

The antioxidant properties of several varieties of *Cichorium* genus vegetables have been attributed, in part, to the presence of phytochemicals including hydroxycinnamic acid derivatives, mono- and diglycosides of flavonoids, and anthocyanins. As reported by Rossetto et al. [6], the presence of these phenolics confers to red chicories an exceptionally high peroxyl radical scavenging activity in terms of both capacity and efficiency, particularly in their early stage of growth. The lower carotenoid values observed in the present study could be due to the limited exposure to sunlight and the lower temperature, during winter, because carotenoids biosynthesis is not stimulated as in vegetables in open fields. As reported by Niizu and Rodriguez-Amaya [34], the green chicory showed a higher average content of 53.7 ± 8.3 (mg/kg) and 35.3 ± 5.0 (mg/kg) for lutein and β-carotene, respectively. As discussed elsewhere [35], green chicory from Lazio exhibited higher carotenoids content. In particular, lutein and β-carotene values were 14.10 ± 3.30 mg/kg and
of direct interaction between epithelial Caco-2 cells and the effect of the antioxidant mixture but indicates the absence of alteration does not specify if these extracts maintain the protective activity on the cellular monolayer by SEM (Figures 4(b) and 5(b)), and the absence of morphological change or extracts activity confirmed by histological analyses (Figures 4(a) and 5(a)) to chemical and physical altered cellular patterns and dramatic relative changes in concentrations of extracts correspond to variations of the chicory extracts concentration. So, small changes in this condition, the epithelial culture is more susceptible to the confluent monolayer of Caco-2 cells at equilibrium. In the intestinal barrier [37]. Our experimental system was a st productive from mucous, contributes to the integrity of the intestinal barrier function in vivo damage. As known, defect in epithelial permeability caused by alteration of TJs is seen in several inflammatory bowel diseases. Different elements, including robust innate immune responses, epithelial paracellular permeability, and epithelial cell integrity, as well as the production of mucus, contribute to the integrity of the glycocalyx, a meshwork of glycoprotein molecules that binds to mucus largely composed of mucopoly saccharides produced by goblet cells. The glycocalyx and mucus form a flexible coat which provides cells protection from mechanical and chemical damage. Nutrients can diffuse into the mucosa, be acted upon by the enzymes in the glycocalyx, and create an area at high concentration of more easily adsorbed molecules by concentration gradient [38]. At the moment, our research is focused on clustering of the food extracts by glycocalyx interaction.

The greatest protective effect on cell cytotoxicity, deriving from Treviso Red radish extract (70 μM) with respect to Variegated Chicory of Castelfranco extract (34 μM), could be due to specific composition in polyphenol-rich fractions. The main flavonoids in above-mentioned extracts are reported in Table 1 and the TJs respond to various naturally plant-derived and food extracts. TEER measurements present dose-response curve patterns, indicating the absence of alteration in Caco-2 cell monolayer, as underlined by our results from 0.2 to 17 μM chicory extracts treatments (Figure 3). Moreover, Cichorium intybus L. seems to counteract and improve the oxidative stress and cellular damage induced by AAPH in vitro Caco-2 cell model (Figure 8). As previously reported [39], when the prooxidant (AAPH) is added, the TEER measurements dramatically decrease until cellular necrosis; in addition, as reported by Finotti et al. [40], the presence of oxidant (AAPH) induces an increase in the mucopolysaccharides secretion located at microvilli glycocalyx. Overall, a better healthy action by Red Chicory of Treviso polyphenol-rich extract on Caco-2 (at 0.2-1.3-10 μM concentrations) could be assumed which does not interact with monolayer cells while as exogenous substance it helps to maintain optimal cellular functions by its strong antioxidant activity.

![Figure 8: TEER changes at different concentration of polyphenol-rich extract after AAPH-induced oxidative stress. (a) Red Chicory of Treviso (RT) extract and (b) Variegated Chicory of Castelfranco (VC) polyphenol extract.](image-url)
On the other hand, Red Chicory is characterized by a high content of anthocyanin pigments [41] that could exert several beneficial health or nutraceutical effects [12, 42, 43].

5. Conclusion

It could be concluded that the TJs response depends on the dose exposure and particular chemical composition of food extracts by synergic and interdependent antioxidant effects (enzymatic and nonenzymatic) with the epithelial glycocalyx. Even if the redox balance does not originate from a single cause, our study suggests that the interaction between antioxidant extracts and the mucopolysaccharide complexes in the glycocalyx could protect the in vivo lining of gut from damage maintaining a healthy and effective intestinal barrier.

Abbreviations

Caco-2: Human colon carcinoma cell line
FRAP: Ferric Reducing Antioxidant Power
TEAC: Trolox Equivalent Antioxidant Capacity
TEER: Transepithelial Electrical Resistance
TJ: Tight-junction
SEM: Scanning Electron Microscopy

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The present study was performed within the Projects BIOVITA and TERRAVITA funded by the Italian Ministry of Agriculture, Food, and Forestry Policies.

References

[1] H. P. Bais and G. A. Ravishankar, "Cichorium intybus L.—cultivation, processing, utility, value addition and biotechnology, with an emphasis on current status and future prospects," Journal of the Science of Food and Agriculture, vol. 81, no. 5, pp. 467–484, 2001.
[2] D. A. Evans, J. B. Hirsch, and S. Dushenkov, "Phenolics, inflammation and nutrigenomics," Journal of the Science of Food and Agriculture, vol. 86, no. 15, pp. 2503–2509, 2006.
[3] M. Gerber, M.-C. Boutron-Ruault, S. Hercberg, E. Riboli, A. Scalbert, and M.-H. Siess, "Food and cancer: state of the art about the protective effect of fruits and vegetables," Bulletin du Cancer, vol. 89, no. 3, pp. 293–312, 2002.
[4] N. Hounsome, B. Hounsome, D. Tomos, and G. Edwards-Jones, "Plant metabolites and nutritional quality of vegetables," Journal of Food Science, vol. 73, no. 4, pp. R48–R65, 2008.
[5] M. Schreiner, "Vegetable crop management strategies to increase the quantity of phytochemicals," European Journal of Nutrition, vol. 44, no. 2, pp. 85–94, 2005.
[6] M. Rossetto, A. Lante, P. Vanzani, P. Spettoli, M. Scarpa, and A. Rigo, "Red chicories as potent scavengers of highly reactive radicals: a study on their phenolic composition and peroxyl radical trapping capacity and efficiency," Journal of Agricultural and Food Chemistry, vol. 53, no. 21, pp. 8169–8175, 2005.
[7] A. Papetti, M. Daglia, and G. Gazzani, “Anti- and pro-oxidant activity of water soluble compounds in Cichorium intybus var. silvestre (Treviso red chicory),” Journal of Pharmaceutical and Biomedical Analysis, vol. 30, no. 4, pp. 939–945, 2002.
[8] G. Gazzani, M. Daglia, A. Papetti, and C. Gregotti, "In vitro and ex vivo anti- and prooxidant components of Cichorium intybus," Journal of Pharmaceutical and Biomedical Analysis, vol. 23, no. 1, pp. 127–133, 2000.
[9] L. Hooper and A. Cassidy, "A review of the health care potential of bioactive compounds," Journal of the Science of Food and Agriculture, vol. 86, no. 12, pp. 1805–1813, 2006.
[10] W. Kalt, C. F. Forney, A. Martin, and R. L. Prior, "Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits," Journal of Agricultural and Food Chemistry, vol. 47, no. 11, pp. 4638–4644, 1999.
[11] L. R. Fukumoto and G. Mazza, "Assessing antioxidant and prooxidant activities of phenolic compounds," Journal of Agricultural and Food Chemistry, vol. 48, no. 8, pp. 3597–3604, 2000.
[12] L. D’Evoli, F. Morroni, G. Lombardi-Boccia et al., "Red chicory (Cichorium intybus L. cultivar) as a potential source of antioxidant anthocyanins for intestinal health," Oxidative Medicine and Cellular Longevity, vol. 2013, Article ID 704310, 8 pages, 2013.
[13] M. G. L. Hertog, P. C. H. Hollman, and D. P. Venema, "Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits," Journal of Agricultural and Food Chemistry, vol. 40, no. 9, pp. 1591–1598, 1992.
[14] E. Azzini, A. Durazzo, M. S. Fodda et al., "Phytochemicals content in Italian garlic bulb (Allium sativum L.) varieties," Journal of Food Research, vol. 3, no. 4, pp. 26–32, 2014.
[15] K. E. Sharpless, M. Arce-Osuna, and J. Brown-Thomas, "Value assignment of retinol, retinyl palmitate, tocopherol, and carotenoid concentrations in Standard Reference Material 2383 (baby food composite)," Journal of AOAC International, vol. 82, no. 2, pp. 288–296, 1999.
[16] G. Maiani, G. Pappalardo, A. Ferro-Luzzi et al., "Accumulation of β-carotene in normal colorectal mucosa and colonic neoplastic lesions in humans," Nutrition and Cancer, vol. 24, no. 1, pp. 23–31, 1995.
[17] I. F. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay," Analytical Biochemistry, vol. 239, no. 1, pp. 70–76, 1996.
[18] N. Pellegrini, D. Del Rio, B. Colombi, M. Bianchi, and F. Brighenti, "Application of the 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages," Journal of Agricultural and Food Chemistry, vol. 51, no. 1, pp. 260–264, 2003.
[19] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, "Antioxidant activity applying an improved ABTS radical cation decolorization assay," Free Radical Biology and Medicine, vol. 26, no. 9–10, pp. 1231–1237, 1999.
[20] S. Ferruzza, G. Ranaldi, M. Di Girolamo, and Y. Sambuy, "The transport of lysine across monolayers of human intestinal epithelial cells (Caco-2) depends on Na⁺-dependent and Na⁺-independent mechanisms on different plasma membrane domains," Journal of Nutrition, vol. 125, no. 10, pp. 2577–2585, 1995.
[21] S. Ferruzza, Y. Sambuy, A. Onetti-Muda, F. Nobili, and M. L. Scarino, "Copper toxicity to tight junctions in the human
intestinal Caco-2 cell line,” in Handbook of Copper Pharmacology and Toxicology, E. J. Massaro, Ed., pp. 397–416, Humana Press, Totowa, NJ, USA, 2002.
[22] T. M. Szczesny, “Holder assembly for ‘Ralph’ type glass knives,” Stain Technology, vol. 53, no. 1, pp. 50–51, 1978.
[23] R. Sembra, “Contributions to semithin sectioning on a conventional rotary microtome,” Stain Technology, vol. 54, no. 5, pp. 251–255, 1979.
[24] L. G. Luna, Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, McGraw Hill Book, New York, NY, USA, 1968.
[25] H. S. Bennett, A. D. Wyrick, S. W. Lee, and J. H. Mcncl Jr., “Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains,” Stain Technology, vol. 51, no. 2, pp. 71–97, 1976.
[26] S. Salvatore, N. Pellegrini, O. V. Brenna et al., “Antioxidant characterization of some Sicilian edible wild greens,” Journal of Agricultural and Food Chemistry, vol. 53, no. 24, pp. 9465–9471, 2005.
[27] C. E. Lewis, J. R. L. Walker, J. E. Lancaster, and K. H. Sutton, “Determination of anthocyanins, flavonoids and phenolic acids in potatoes. II: wild, tuberous Solanum species,” Journal of the Science of Food and Agriculture, vol. 77, no. 1, pp. 58–63, 1998.
[28] R. Chirinos, D. Campos, C. Arbizu et al., “Effect of genotype, maturity stage and post-harvest storage on phenolic compounds, carotenoid content and antioxidant capacity, of Andean mashua tubers (Tropaeolum tuberosum Ruiz & Pavón),” Journal of the Science of Food and Agriculture, vol. 87, no. 3, pp. 437–446, 2007.
[29] A. Bhattacharyya, R. Chattopadhyay, S. Mitra, and S. E. Crowe, “Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases,” Physiological Reviews, vol. 94, no. 2, pp. 329–354, 2014.
[30] C. Cavin, M. Delannoy, A. Malnoe et al., “Inhibition of the expression and activity of cyclooxygenase-2 by chicory extract,” Biochemical and Biophysical Research Communications, vol. 327, no. 3, pp. 742–749, 2005.
[31] H. A. Hassan and M. I. Yousef, “Ameliorating effect of chicory (Cichorium intybus L.)-supplemented diet against nitrosamine precursors-induced liver injury and oxidative stress in male rats,” Food and Chemical Toxicology, vol. 48, no. 8-9, pp. 2163–2169, 2010.
[32] C. Masungi, J. Mensch, B. Willems et al., “Usefulness of a novel Caco-2 cell perfusion system II. Characterization of monolayer properties and peptidase activity,” Pharmazie, vol. 64, no. 1, pp. 36–42, 2009.
[33] C. Jonker-Venter, D. Snyman, C. Janse van Rensburg et al., “Low molecular weight quaternised chitosan (II): in vitro assessment of absorption enhancing properties,” Pharmazie, vol. 61, no. 4, pp. 301–305, 2006.
[34] P. Y. Niizu and D. B. Rodriguez-Amaya, “New data on the carotenoid composition of raw salad vegetables,” Journal of Food Composition and Analysis, vol. 18, no. 8, pp. 739–749, 2005.
[35] E. Azzini, A. Durazzo, M. S. Foddai, E. Venneria, A. Ruguzzini, and G. Maiani, “Biodiversity and local food products in Italy,” Journal of Agriculture and Biodiversity Research, vol. 1, no. 1, pp. 1–10, 2012.
[36] N. Pellegrini, M. Serafini, B. Colombi et al., “Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays,” Journal of Nutrition, vol. 133, no. 9, pp. 2812–2819, 2003.