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Antioxidant Compounds and Antioxidant Activity in "Early Potatoes"

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The antioxidant content and the antioxidant capacity of both hydrophilic and lipophilic antioxidant extracts from four "early potato" cultivars, grown in two different locations (Racale and Monteroni), were examined. There was a considerable variation in carotenoid content and weak differences in the ascorbic acid concentration of the examined cultivars of "early potato" and between the harvested locations. An increase in both methanol/water (8:2 v/v) and phosphate buffer soluble (PBS) free phenols (70%) and bound phenols (28%) in the extracts from the cultivars grown at Racale site was found and discussed. Examination of individual phenols revealed that chlorogenic acid and catechin were the major phenols present in potato tuber extracts; a moderate amount of caffeic acid and ferulic acid was also detected. The total equivalent antioxidant capacity (TEAC) was higher in the Racale extracts and a highly positive linear relationship ($R^2 = 0.8193$) between TEAC values and total phenolic content was observed. The oxyradical scavenging capacity (TOSC) of methanol/water and PBS extracts of peel and whole potatoes against the reactive oxygen species (ROS) peroxyl radicals, peroxynitrite, and hydroxyl radicals was also analyzed. A highly significant linear correlation ($R^2 = 0.9613$) between total antioxidant capacity (as a sum of peroxyl radicals + peroxynitrite) and total phenol content of methanol/water extracts was established. Moreover, proliferation of human mammalian cancer (MCF-7) cells was significantly inhibited in a dose-dependent manner after exposure to potato extracts. These data can be useful for "early potato" tuber characterization and suggest that the "early potato" has a potential as a dietary source of antioxidants.

KEYWORDS: Potatoes; ascorbic acid; phenols; antioxidant activity; antiproliferative activity

INTRODUCTION

The potato (*Solanum tuberosum* L.) is the world's most widely grown crop and the fourth largest in terms of fresh produce after rice, wheat, and maize. It represents the staple source of nutrients and energy in many different countries. The extensive geographical distribution of potatoes indicates a wide ecological diversity and a range of physiological adaptations to extremes of temperature and humidity. In Italy, the potato crop is the third most important vegetable crop (after tomatoes and artichokes). National potato production is based mainly on two varieties of potato: the "early potato" (about 30% of the total marketed produce) harvested before June, and the "common potato" (70% of the total marketed produce) with harvest starting from the middle of June. "Early potatoes" are defined as "potatoes harvested before they are completely mature, marketed immediately after harvesting and whose skin can be easily removed without peeling" (UNECE of Geneva, FFV-30/2001). "Early potato" production is typical of some areas of Southern Italy, particularly Puglia, Campania, and Sicily regions, where over 90% of the total national production of early potatoes is grown. The Puglia region and above all the areas between the Adriatic and Ionian Seas are characterized by mild winters, during which temperatures rarely drop below 0 °C. These favorable conditions are ideal for "early potato" cultivation. Recently, in Puglia new experimental and extension activities were developed to introduce new genotypes, to study the influence of some innovative agronomic techniques, and to improve the qualitative, nutritional, and organoleptic characteristics of early potatoes.

Potatoes are a proven source of quality proteins, carbohydrates, and minerals such as calcium, potassium, and phosphorus, and their value within the human diet is often underestimated or ignored (*1*). Potatoes not only contain a great variety of nutrients, but also have many small molecular weight compounds whose many beneficial effects on health have been recognized (*2*). The health benefits of these molecular weight

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components are believed to be attributable to the structure of diverse groups of phytochemicals and vitamins (*3*). For example, potatoes accrue a great variety of secondary metabolites such as flavonoids (including compounds such as flavonols, kaemferol, quercitin, myricetin, etc.) and carotenoids (*2, 4, 5*). Phenol compounds (including compounds such as chlorogenic, catechin, *p*-coumaric, ferulic, and caffeic acids) are also reported to be present in potato tubers at relevant concentrations (*3, 6*) and are considered an integral part of the human diet and a good source for medicinal preparations. These compounds are present in potatoes as both free and bound forms. Bound phenols, mainly in the form of a-glycosides, may survive digestion in the human stomach and small intestine and may remain intact to reach the colon. Here they are released and exert their bioactivity. Similar to phenol compounds, potato vitamins have been reported to have several important roles in human health and nutrition, and in food chemistry (*7, 8*). As reported by other authors (*9*) potato tubers also contain L-ascorbic acid and dehydroascorbic acid, and both acids are influenced by several factors including cultivar, production practices, and harvest and storage conditions. Potato antioxidant compounds are closely associated with a strong antioxidant capacity (*3*) and their antioxidant effect is mainly due to their redox properties and is the result of various possible mechanisms: free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity. The antioxidant compounds may act cooperatively, thereby providing organisms with greater protection than would be expected by the contribution of a single antioxidant acting alone. Synergistically or additively, dietary antioxidants provide bioactive mechanisms to reduce free radical activity induced by oxidative stress (*10*). It might be of interest to know why there is a general growing concern in the antioxidant properties of the "early potato". Thus, analytical and compositional aspects of antioxidant compounds in "early potatoes" must be thoroughly investigated and discussed. Moreover, because of the wide range of antioxidant concentrations reported in potatoes (*3*), it is likely that its antioxidant capacity might vary according to variety, growing season, environmental and climatic conditions, plant disease, soil type and geographic locations.

In this study, four "early potato" varieties grown in two different types of soil were selected on the basis of previous experiments conducted in the southern area of Italy (*11*). The objective was to identify and quantify the groups of antioxidants in potato tubers and to measure the total antioxidant activity of hydrophilic and lipophilic antioxidants by means of their total oxyradical scavenging capacity (*12, 13*). In particular, the antioxidant capacity of potato extracts against three reactive oxygen species such as peroxynitrite and peroxyl or hydroxyl radicals was investigated (*14, 15*). This study also examined antiproliferative activity and the effect on viability of potato extracts in human mammalian cancer cell (MCF-7) in vitro.

MATERIALS AND METHODS

Instruments. A high-speed Waring blender (Torrington, CO, USA) and a FreeZone 2.5 LabConco lyophilizer (Kansas City, MO, USA) for sample preparation, a Beckman-Coulter DU-640 spectrophotometer for protein and TEAC analyses, a Beckman-Coulter 126 HPLC System Gold equipped with a Beckman 168 diode array detector and an autosampler Beckman System Gold 508 (Fullerton, CA, USA) for ascorbic acid and phenol acid evaluation, and a Shimadzu GC-17A gas chromatograph (Kyoto, Japan) equipped with a flame ionization detector (FID) for TOSC analysis were used.

Reagents and Standards. Methanol, ethanol and acetonitrile (HPLC grade), and acetic and phosphoric acids were purchased from Merck Darmstadt, Germany; metaphosphoric acid, ascorbic acid, caffeic acid, ferulic acid, coumaric acid, chlorogenic acid, 2,29-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate (dipotassium peroxdisulfate), RPMI 1640 medium, Dulbecco's phosphate buffered saline (PBS), L-glutamine (GLN), antibiotic-antimycotic stabilized solution (AA), heat inactivated fetal bovine serum (FBS), trypan blue solution 0.4%, trypsin-EDTA solution, 3-morpholino-sydnonymide (SIN-1), and 2,2′-azobis(amidinopropane) (ABAP) were purchased from Sigma-Aldrich, Milan, Italy; trolox was purchased from Hoffman-La Roche; 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid was purchased from Aldrich Chemical Co., Gillingham, Dorset, UK; and CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay (MTS) was purchased from Promega Italia srl, Milan, Italy.

Material and Sample Preparation. Four cultivars of "early potatoes" were randomly grown in two experimental fields showing some pedological and climatic differences, at Monteroni and Racale, in the Puglia Region, Italy, in 2005 and 2006 as previously reported (*11*). One experiment was performed in an experimental farm at Monteroni in a silt loam soil, with 5% sand >50 μm, 32% sand <50 μm, 37% silt (2−50 μm), 12% clay (<2 μm), 11% $CaCO_3$, 1.3% organic matter, 6.7 mg 100 g^{-1} soil phosphorus, and 8.3 pH KCl. A parallel experiment was performed in an experimental farm at Racale in a silt loam soil, with 8% sand >50 μm, 30% sand <50 μm, 27% silt (2−50 μm), 15% clay (<2 μm), 18% $CaCO_3$, 0.9% organic matter, 2.3 mg 100 g^{-1} soil phosphorus, and 8.0 pH KCl (soil sampling before fertilization). The Racale farm is situated close to the Adriatic coast, while the Monteroni farm is located about 25 Km from the coast. Fertilization was 75:150:80 N:K_2O:P_2O_5 kg ha^{-1}, respectively. Each batch consisted of three replicated plots of each cultivar/genotype arranged in a randomized block design. Potato seeds were the commercial Sieglinde Francese (Sieglinde F) and Nicola cultivars, and ISCI 4052 (Rubino) and ISCI 67 cultivars from Italian CRA collections. Seeds were planted at the end of February and 18 weeks after planting the tubers were carefully harvested by hand, then washed, dried, and placed into storage under reduced light at 4−6 °C and 70−90% relative humidity. Four weeks after storage a selected sample of 30 tubers was taken for biochemical analyses. For each sample, tubers were dissected longitudinally in slices and samples of flesh + skin (whole potato) or skin (peel, 0.5−1 mm) of each tuber, manually diced, were immediately frozen in liquid nitrogen, ground in a laboratory Waring blender, and lyophilized under vacuum, then the powder was stored at −20 °C prior to analyses. Lyophilization of the samples was assumed to be the most cautious method for isolation of antioxidant compounds, and it was preferred to other less cautious methods for homogenization of fresh samples.

Determination of Ascorbic Acid Content. Ascorbic acid was extracted from potato powder (2 g) by using 10 mL of a 5% (w/v) aqueous solution of metaphosphoric acid containing 1% (w/v) dithiothreitol (*16*). Extracts were separated by reverse-phase HPLC, using a NH_2 Waters Spherisorb 4.6 × 250 mm (Waters Corporation, Milford, MA) and analyzed at 254 nm wavelengths. Separations were achieved by utilizing a mobile phase of acidified distilled water (0.1% phosphoric acid) and acetonitrile at a ratio of 25:75 and a flow rate of 1 mL/min (*9*). Quantification was achieved by comparing the sample peak area with those of known amounts of ascorbic acid standard compound and expressed as mg 100 g^{-1} of dry weigh (dw).

Protein Determination. The amount of soluble proteins was determined in triplicate with use of freeze-dried potato powders (3 g) dispersed in a 10 mM sodium phosphate buffer, pH 7 (PBS), containing 15% polyvinylpolypyrrolidone (PVPP) and 10 mL of a protease inhibitor cocktail (Sigma-Aldric, Milan, Italy). The sample was homogenized at 5 °C, centrifuged, and filtered through cheesecloth. The filtrate was added to an equal volume of a cold methanol−water (5:1 v/v) solution containing 0.1 M ammonium acetate. The proteins were precipitated at −20 °C and collected by centrifugation, washed twice with acetone, and the resulting pellet was suspended in a PBS buffer and used for the protein assay. Protein concentration was estimated with the Bio-Rad protein dye binding assay (Bio-Rad Laboratories, Mississauga, ON, Canada) (*17*), according to manufacturer's instructions (expressed as mg g^{-1} of dry weight).

Total Starch and Amylose Determination. Starch and amylose content were determined according to the standard procedure described

in Megazyme Assay Kit (K-TSTA and K-AMYL, Megazyme International Ireland ltd., Bray, Co. Wichlow, Ireland). The evaluated starch content was expressed as g 100 g^{-1} of dry weight. The amylose content was expressed as percentage of total starch.

Carotenoid Determination. Total carotenoids were extracted by acetone−ethanol (1:1 v/v) and analyzed spectrophotometrically (*18*). Briefly, a 2-g potato powder sample was homogenized with acetone−ethanol (1:1) solution containing 200 mg L^{-1} BHT in a Waring blender; the homogenate was filtered through cheesecloth and solvent was added to the final volume of 100 mL. The extracts were mixed with 50 mL of hexane followed by the addition of 25 mL of distilled water. The samples were mixed thoroughly and then allowed to stand for 30 min. The hexane layer was collected and the absorbance measured at 470 nm. Concentration was expressed as mg of β-carotene equivalents 100 g^{-1} dry weight.

Phenolic Acids Determination. To extract the free phenol compounds, potato powders (2 g) were homogenized with an 8:2 (v/v) methanol/water solution (methanol/water) and centrifuged. The residue was treated with methanol/water for two successive extractions. The supernatants were combined, centrifuged, and concentrated with a rotary evaporator and used for analysis. To extract conjugated phenols the residues of the potato power were treated with 4 M NaOH for 1 h at room temperature and then centrifuged. The residue was separated from the supernatant and used for two successive extractions with the same amount of NaOH. All the supernatants were combined and acidified with HCl and extracted three times with ethyl acetate, then the solution was evaporated to dryness with a rotary evaporator. The residue containing the bound-form phenolics was dissolved in a methanol/water (1:1 v/v) solution and used for phenol analyses (*19, 20*). The soluble phenols extracted in a 10 mM sodium phosphate buffer, pH 7 (PBS), using freeze-dried potato powders (2 g), were homogenized in a Waring blender, filtered through a Millipore Ultrafree-15 filter (Millipore, Bedford, MA, USA), and centrifuged, then the supernatant was used for the successive analyses. Total phenols were estimated by using the Folin−Ciocalteu colorimetric method (*21*). Total phenol content was standardized against gallic acid and expressed as milligrams of gallic acid equivalents 100 g^{-1} of dry weight. The individual phenol was identified and quantified with HPLC by comparison with an external standard of corresponding known phenol and expressed as mg 100 g^{-1} of dry weight (*16, 20*). Separation was carried out with a reversed phase RP-C18 Beckman Ultrasfere 4.6 × 250 mm column (Beckman-Coulter, Fullerton, CA, USA) and isocratic elution with methanol/water/acetic acid (34:65:1, v/v) at a flow rate of 1.0 mL/min. Chromatographic peaks were identified at 280 and 325 nm by comparing the retention time of the sample chromatographic peaks with those of standard compounds, using a diode-array detector.

Antioxidant Activity Analysis. Evaluation of antioxidant capacity of potato extracts was determined by the TEAC and TOSC methods (*12, 14*). The $ABTS^{\bullet}$ assay, based on the measurement of a range of end points at a fixed time activation, was used for the screening of antioxidant activity of both lipophilic and hydrophilic extracts (*12*). Briefly, a mixture of 7 mM ABTS and 4.95 M potassium persulfate (1:1, v/v) was left to stand for 16 h at room temperature in the dark to form radical cation $ABTS^{\bullet+}$. The solution was diluted to absorbance values of 0.70 at 734 nm with ethanol or phosphate buffer solution depending on the solvent used for the extracts. Standards or plant extracts were diluted with methanol/water or PBS to give 20−80% inhibition of the blank absorbance with 10 μL of the sample. A trolox calibration curve of 2.5−30 μM was prepared under the same conditions as above. The antioxidant capacity of the samples was calculated, based on the inhibition exerted by standard trolox concentrations at 734 nm after 6 min. Results were expressed in terms of mmol of trolox equivalents g^{-1} dry weight (TEAC) of the potato powder. The peroxyl radical, hydroxyl radical, and peroxynitrite scavenging activity (TOSC) were quantified following the method previously described (*14*). Reactions were carried out in 10 mL rubber sealed vials in final volume of 1 mL. The analyses of ethylene were carried out with a GC-17A gas chromatograph, using a Restek T-Alumina column (30 m × 0.53 mm; Supercrom srl, Milan, Italy). The degree of inhibition of ethylene formation from KMBA in the presence of antioxidants was quantified according to the equation TOSC = 100 − (SA/CA × 100), where SA and CA are the integrated areas from the curve that best define the experimental points during the reaction time course for sample and control reactions, respectively. A TOSC value of 0 corresponds to a sample that displays no scavenging capacity, a solution that suppresses the ethylene formation completely achieves a TOSC value of 100% (*13*). The capacities of the different potato extracts to inhibit ethylene production were tested toward peroxyl and hydroxyl radicals and peroxynitrite. The experimental TOSC values for potato extracts were plotted against the reciprocal value of the dilution factor of the added extracts. The TOSC value at 20%, 50%, and 80% is expressed as micromoles of gallic acid equivalents (*14, 15*). The specific Total Oxyradical Scavenging Capacity was calculated by dividing the experiential TOSC value by mg dry weight potato (*13*).

Cell Proliferation and Viability Analysis. Breast cancer cell line MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, UK). The cell line was routinely grown in RPMI-1640 medium containing 10% (v/v) heat inactivated fetal bovine serum (FBS), 1% GLN, and 1% AA in 25 cm^2 plastic flasks (Iwaki, Japan) at 37 °C in a 5% CO_2 humidified atmosphere. Cells were passaged at 70−80% confluence, about twice a week by trypsinization (*22*). The methanol/water potato extracts were filtered through 0.22 μm Millex-HV (Millipore), diluted with RPMI medium, and added to the cell culture at a concentration ranging from 3.5×10^{-6} to 10^{-3} μg of gallic acid equivalents μL^{-1}. The final level of methanol in culture medium never exceeded 4% (v/v). Cell proliferation was measured by using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS) and an electron coupling reagent (phenazine methosulfate, PMS) following the procedure described by the manufacturer. Treated cell viability was expressed as a percentage of control cells. All determinations were carried out in quadruplicate. Dose−response curves were plotted against the mean values calculated as percentages of untreated samples. Cell viability was assessed by Trypan blue.

Statistical Analysis. In this study, all determinations were conducted a minimum of three times, and results were calculated as mean ± standard deviation (SD). The results were compared by analysis of variance (ANOVA) with Minitab software (Minitab, Inc., State College, PA). Pairwise multiple comparisons were done by Tukey's significant difference test with the family error rate held at 0.05. Simple linear correlation analysis was used to indicate the measure of the correlation and the strength of the relationship between two variables.

RESULTS AND DISCUSSION

The data across the 2 years obtained from the four cultivars of "early potato" showed relatively few statistically significant differences. To facilitate the presentation and discussion of the data, the results obtained from potato tubers grown during the second experimental year, the 2006 season, are reported and discussed here. The values reported in the figures and tables are ranked in two groups depending on the growing site, Monteroni and Racale. The results obtained from the yield and biometric parameters showed that the potato crops at the Racale site had higher yield and higher vegetative growth (data not shown) than those cultivated at the Monteroni site (*11*). A possible explanation of these results is the different composition of the soil in the Racale field and, particularly, specific meteorological factors. Night temperatures during the winter−spring period (potato growing season) in 2005 and 2006 were never below 7 °C in Racale (sea influence), while they were around 0 °C for several nights in Monteroni.

Chemical Analysis. The dry matter of "early potato" from the two different locations varied in a relatively small range from 17 to 20%. The mean value of dry matter proved to be different when cultivars of the two different locations were compared. A higher value of dry matter was observed for all the cultivars cultivated in Monteroni compared with the same cultivars in Racale with a mean increase of about 10%. Carbohydrate accumulation represented the main contribution

Table 1. Protein Contents, Starch and Amylose, Carotenoids, and Ascorbic Acid in Whole Potato Tubers[a]

cultivar	field	proteins (mg g^{-1} dw)	starch (g 100 g^{-1} dw)	amylose (%)	carotenoids (mg 100 g^{-1} dw)	ascorbic acid (mg 100 g^{-1} dw)
Nicola	Monteroni	8.2 ± 0.8 a	45 ± 0.4 f	18	0.24 ± 0.03 b	93 ± 8 a
Sieglinde F	Monteroni	10 ± 0.9 a	52 ± 0.4 g	17	0.27 ± 0.03 b	82 ± 5 a
Isci 4052	Monteroni	7.9 ± 0.6 a	41 ± 1 d	19	0.14 ± 0.01 a	48 ± 5 b
Isci 67	Monteroni	8.6 ± 0.6 a	42 ± 0.4 de	14	0.13 ± 0.01 a	55 ± 5 b
Nicola	Racale	9.8 ± 0.7 a	41 ± 0.2 d	19	0.33 ± 0.03 b	98 ± 7 a
Sieglinde F	Racale	14 ± 0.9 c	40 ± 0.3 c	15	0.30 ± 0.01 b	101 ± 9 a
Isci 4052	Racale	10 ± 0.7 a	33 ± 0.4 ab	19	0.16 ± 0.02 a	60 ± 5 b
Isci 67	Racale	12 ± 1 b	32 ± 0.3 a	14	0.17 ± 0.02 a	56 ± 4 b

[a] Mean ± SD ($n = 6$). Mean values within a column with different letters are significantly different at $P < 0.05$.

Table 2. Total Free and Bound Phenol Contents of Methanol−Water (8:2 m/w) and PBS Extracts from Whole Potato Tubers and Peels[a]

		total free phenols (mg 100 g^{-1} dw)				total bound phenols (mg 100 g^{-1} dw)	
		m/w extracts		PBS extracts		m/w extracts	
cultivar	field	whole	peel	whole	peel	whole	peel
Nicola	Monteroni	165 ± 9 c	312 ± 8 b	60 ± 0.1 b	123 ± 2 a	14 ± 0.2 a	10 ± 0.2 a
Sieglinde F	Monteroni	107 ± 5 b	227 ± 18 a	46 ± 0.4 a	132 ± 2 a	11 ± 0.1 a	10 ± 0.7 a
Isci 4052	Monteroni	172 ± 7 c	221 ± 16 a	78 ± 0.1 c	156 ± 2b	13 ± 1 a	13 ± 1 b
Isci 67	Monteroni	95 ± 4 a	315 ± 25 b	44 ± 0.7 a	137 ± 3 a	14 ± 0.3 a	14 ± 0.6 b
Nicola	Racale	289 ± 6 f	427 ± 10 c	132 ± 1.2 e	242 ± 10 d	18 ± 0.3 b	12 ± 0.3 b
Sieglinde F	Racale	194 ± 12 d	301 ± 30 b	88 ± 0.2 c	182 ± 4 c	12 ± 0.1 a	14 ± 0.7 b
Isci 4052	Racale	264 ± 3 e	288 ± 24 b	97 ± 0.8 d	197 ± 12 c	15 ± 0.4 b	16 ± 1 c
Isci 67	Racale	159 ± 10 c	339 ± 32 b	80 ± 0.4 c	189 ± 2 c	23 ± 1 c	16 ± 1 c

[a] Mean ± SD ($n = 6$). Mean values within a column with different letters are significantly different at $P < 0.05$.

Table 3. Major Components of Free Phenols Extracted from Whole Potato Tubers[a]

		whole free phenols (mg 100 g^{-1} dw)					
		methanol/water extracts				PBS extracts	
cultivar	field	catechin	clorogenic acid	caffeic acid	ferulic acid	catechin	ferulic acid
Nicola	Monteroni	66 ± 4 a	92 ± 5 b	8 ± 0.2 a	1.2 ± 0.1 b	54 ± 2 a	0.9 ± 0.1 a
Sieglinde F	Monteroni	43 ± 4 a	53 ± 5 a	11 ± 0.4 b	0.6 ± 0.1 a	49 ± 2 a	0.8 ± 0.1 a
Isci 4052	Monteroni	91 ± 7 b	57 ± 2 a	5 ± 0.3 a	1.9 ± 0.2 b	68 ± 3 ab	1.8 ± 0.2 b
Isci 67	Monteroni	65 ± 6 a	47 ± 4 a	7 ± 0.8 a	0.7 ± 0.1 a	45 ± 4 a	1.2 ± 0.1 ab
Nicola	Racale	157 ± 7 c	92 ± 5 b	10 ± 0.7 b	1.9 ± 0.3 b	117 ± 6 bc	2.0 ± 0.1 b
Sieglinde F	Racale	91 ± 6 b	79 ± 4 a	12 ± 1 b	1.1 ± 0.3 b	98 ± 3 b	1.5 ± 0.2 ab
Isci 4052	Racale	154 ± 2 c	82 ± 4 b	8 ± 0.7 a	3.9 ± 0.4 c	132 ± 6 c	1.6 ± 0.5 ab
Isci 67	Racale	110 ± 3.5 b	61 ± 3 a	9 ± 0.6 a	1.7 ± 0.2 b	94 ± 4 b	2.2 ± 0.2 b

[a] Mean ± SD ($n = 6$). Mean values within a column with different letters are significantly different at $P < 0.05$.

to the dry matter. Starch content of the potatoes cultivated in Monteroni was slightly higher ($P < 0.05$) when compared with ranges reported for those cultivated in Racale. The protein of the potato cultivars grown in Racale site showed a 20% increase in protein content compared with those cultivated in Monteroni (**Table 1**). The "early potato" revealed a considerable variation of carotenoid content in their yellow-fleshed pulp within the cultivar ($P < 0.05$) examined: carotenoid concentration ranged between 0.13 and 0.28 mg 100 g^{-1} of dw in the extracts obtained from the Monteroni field and 0.16 and 0.33 mg 100 g^{-1} of dw in those obtained from the Racale field. Nicola potato extracts had the maximum carotenoid content while ISCI 4052 had the minimum of all varieties tested in both fields (**Table 1**).

The mean of ascorbic acid content presented in **Table 1** illustrates the major differences among "early potato" cultivars. Ascorbic acid in ISCI 4052 and ISCI 67 (48−60 and 55−56 mg 100 g^{-1} of dw, respectively) was lower than that in Nicola and Sieglinde F (93−98 and 82−101100 g^{-1} of dw, respectively). Considering the varieties grown in each area together as one group, there was a weak variation in ascorbic acid content between the group of varieties at Monteroni and those at the Racale site. This large variability in ascorbic acid content among the cultivars indicates that the trait is related to genetics, while examination of data from the cultivation sites indicates only a moderate increase in all the cultivars grown in Racale compared with those cultivated in Monteroni. In general, protein carbohydrate, ascorbic acid, and carotenoid level detected in this study are in agreement with levels previously reported in other potato cultivars as well as the variability within different cultivation sites (*3, 23–25*).

The content of phenols in the free and bound form in whole and peel potato is presented in **Tables 2–4**. Considering the whole potato tuber (flesh + peel), the content of free phenols was much greater than the bound forms in all the cultivars examined. The bound form accounted for 5−7% of total phenol content in the whole tuber. In **Table 2**, substantial differences are evident between the overall mean content of methanol/water free phenols and those that are PBS-soluble. The concentration of methanol/water phenols was almost twice that of the PBS-soluble phenols in the whole tubers harvested from both fields. Comparison of the value of each class of phenols for each individual cultivar grown in the same area indicated a significant difference ($P < 0.05$) among the cultivars. Tubers cultivated in Monteroni contained from 90 to 170 mg 100 g^{-1} dw lipophilic free phenols and from 40 to 70 mg 100 g^{-1} dw hydrophilic free phenols, while the same cultivars grown in Racale ranged from 150 to 280 mg 100 g^{-1} dw for methanol/water soluble-free phenols and 80 to 130 mg 100 g^{-1} dw for PBS-

Table 4. Major Free Phenol Components in Methanol and PBS Extracts from Potato Peels[a]

		peel phenol compounds					
		methanol/water extracts					PBS extracts
cultivar	field	catechin	clorogenic acid	caffeic acid	coumaric acid	ferulic acid	catechin
Nicola	Monteroni	123 ± 14 b	283 ± 4 c	40 ± 2 b	nd	9 ± 0.05 e	81 ± 3 a
Sieglinde F	Monteroni	84 ± 8 a	99 ± 8 a	25 ± 4 a	0.4 ± 0.1 a	3 ± 0.5 a	80 ± 6 a
Isci 4052	Monteroni	95 ± 12 a	98 ± 9 a	37 ± 2 b	0.8 ± 0.1 b	3 ± 0.5 a	87 ± 8 a
Isci 67	Monteroni	111 ± 11 b	147 ± 1 b	21 ± 0 a	0.7 ± 0.4 c	3 ± 0.5 a	86 ± 8 a
Nicola	Racale	204 ± 14 d	246 ± 6 c	41 ± 4 b	nd	7 ± 1 d	137 ± 14 c
Sieglinde F	Racale	113 ± 9 b	139 ± 9 b	35 ± 5 b	9.2 ± 0 e	5 ± 1 b	123 ± 2 b
Isci 4052	Racale	118 ± 8 b	128 ± 10 b	50 ± 5 c	nd	4 ± 0 a	118 ± 2 b
Isci 67	Racale	148 ± 2 c	184 ± 14 c	29 ± 7 a	2.1 ± 0.5 d	2 ± 1 a	111 ± 5 b

[a] Mean ± SD ($n = 6$). Mean values within a column with different letters are significantly different at $P < 0.05$. nd: not detected.

soluble ones. The average value of the total free phenols in the extracts (**Table 2**) showed a significant increase in the group of the four cultivars cultivated in Racale, on the order of 70%, for both lipophilic and hydrophilic extracts, when compared with the group grown in Monteroni. Data from the methanol/water extracts showed that all the cultivars had high concentrations of free chlorogenic acid and the flavonoid catechin, moderate amounts of caffeic acid together with lower concentrations of ferulic acid, and traces of *p*-coumaric acid and other unidentified peaks as appeared in the HPLC chromatogram. In potato PBS extracts, the major phenols were identified as flavonoid catechin (40−130 mg 100 g^{-1} of dw) and, in a small amount, ferulic acid (**Table 3**). The sum of catechin plus chlorogenic acid represented >90% of the total phenol compounds present in "early potato" tubers. This agrees with previous studies, which indicated chlorogenic acid and catechin (*6, 26, 27*) as the main phenol compounds in potatoes.

The concentration of bound phenols in the whole tubers of "early potato" proved to be less concentrated than free phenols and ranged between 11 and 23 mg 100 g^{-1} of dw. Comparing the concentration of bound phenols of each cultivar in the two different areas, an incremental trend (28%) of bound phenols in favor of all cultivars grown in Racale (**Table 2**) was observed. Catechin was the major compound present in bound form, with a concentration of up to 20 mg 100 g^{-1} of dw, followed by moderate amounts of clorogenic acid, caffeic acid, and ferulic acid, with actual concentrations depending on the cultivar.

Selected small size "early potatoes" (20−40 mm diameter) are commonly sold in the market and are eaten baked without peeling. For this reason the content and the activity of potato peels was separately analyzed to calculate the intake of peel antioxidants on the total content.

Potato peels are reported to have high antioxidant activity due to the presence of chlorogenic, gallic, cinnamic, and ferulic acids as the major antioxidant compounds in the extract (*28*). In the "early potato", the total amount of free phenols detected in the peel also varied between the four cultivars examined. Significantly, higher values ($P < 0.05$) of free phenols, both methanol/water and PBS extracted, were found in peel when compared to the whole tuber and with a concentration in favor of the cultivar grown in Racale. In the peel extracts, chlorogenic acid and caffeic acid accounted for 40−50% and 8−16% of the total phenol acid content, respectively; additionally, more catechin was present at a high concentration (35−40%) while *p*-coumaric and ferulic acid were present at a lower concentration. In addition, an isomer of chlorogenic acid, presumably neochlorogenic acid, was identified in the ISCI 67 extracts. The concentrations of total free and bound phenols observed in "early potato" were consistent with the concentrations reviewed by Brown (*3*). Previous studies on the phenolic composition of potatoes have reported high concentrations of chlorogenic acid and lower amounts of caffeic, vanillic, sinapic, gallic, syringic, *p*-coumaric, and cinnamic acid (*4, 28*), while other authors (*4, 6, 29*) reported chlorogenic acid, catechin, and *p*-coumaric acids as a major constituent in the peels and/or flesh of potato tuber cultivars. These discrepancies could be due to different causes such as sites of production and climatic conditions associated, maybe, with different pathogens, harvesting periods, and cultivar used.

Table 5. Total Antioxidant Capacity (TEAC) of Different Early Potato Extracts[a]

		TEAC (mmol Trolox equivalents g^{-1} dw)		
cultivar	field	methanol/water x extracts	PBS extracts	ascorbic acid extracts
Nicola	Monteroni	11 ± 1b	12 ± 0.6a	12 ± 0.1b
Sieglinde F	Monteroni	6.9 ± 1 a	13 ± 2 a	9.9 ± 0.7 a
Isci 4052	Monteroni	6.8 ± 0.4 a	28 ± 3 b	12 ± 0.8 b
Isci 67	Monteroni	6.9 ± 2 a	14 ± 4 a	9.5 ± 1 a
Nicola	Racale	11 ± 0.6 b	32 ± 2 c	18 ± 1 d
Sieglinde F	Racale	11 ± 0.5 b	25 ± 1 b	17 ± 0.1 c
Isci 4052	Racale	8.9 ± 0.8 b	47 ± 1 d	20 ± 0.9 d
Isci 67	Racale	9.3 ± 1 b	27 ± 2 b	15 ± 0.6 c

[a] Mean ± SD ($n = 6$). Mean values within a column with different letters are significantly different at $P < 0.05$.

Antioxidant Activity. The total antioxidant capacity of potato extracts is reported in **Table 5**. The trend observed for both ascorbic acid and PBS extracts was an increased antioxidant capacity for the cultivars grown in Racale. The mean value of ascorbic acid in extracts from Racale was 18 TEAC, with ISCI 4052 exhibiting the strongest radical scavenging capacity (20 TEAC), and ISCI 67 the lowest one (15 TEAC) (**Table 5**). Taking into account the TEAC average values of the four cultivars for each location, a positive correlation with ascorbic acid content of "early potato" was observed. A discrete correlation ($R^2 = 0.5537$) between TEAC values and PBS phenol extracts was also observed. Nicola and ISCI 4052 cultivars grown in the Racale site had the highest TEAC value (**Table 5**). The activity of hydrophilic antioxidants might be largely related to the content of water soluble phenols present in the extracts. The data reported here clearly indicate that higher catechin content in the PBS extracts corresponds to higher TEAC values, while lower catechin concentration in PBS extracts reflects a decreased TEAC value. This result agrees with other reports (*3, 30*) which indicated that potatoes contain water-soluble phenol antioxidants that act as radical scavengers. In summary, the hydrophilic fractions (ascorbic acid + PBS extracts) in potato extracts have a high TEAC activity and make up 68% of the total antioxidant capacity of the whole extract. A relevant antioxidant activity (30% of total activity) in the

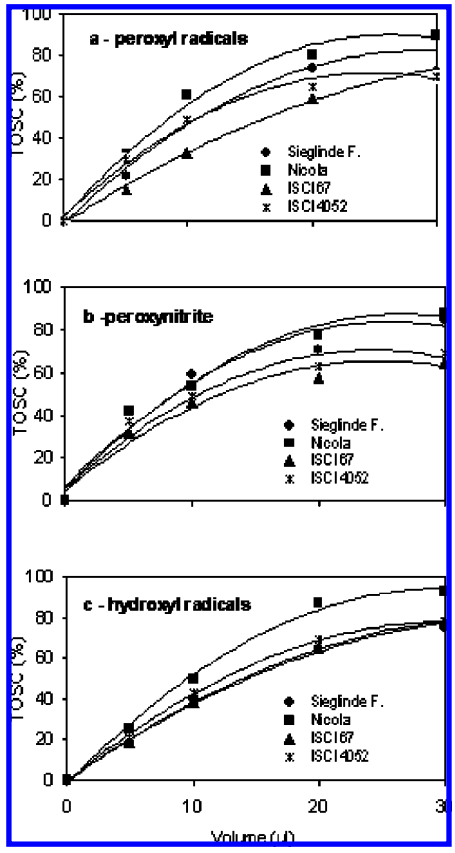

Figure 1. Characteristic dose−response of "early potato" extracts against peroxyl radicals, peroxynitrite, and hydroxyl radicals.

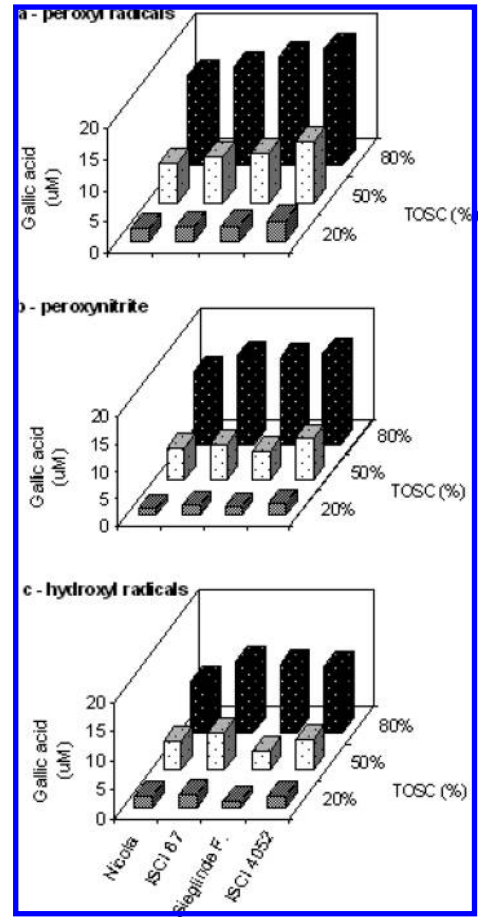

Figure 2. Concentrations of potato antioxidants, as μM of gallic acid equivalents, in "early potato" extracts against peroxyl radicals, peroxynitrite, and hydroxyl radicals for different TOSC values (20%, 50%, and 80%).

methanol/water extracts of "early potatoes" was also observed. The total antioxidant activity (7−11 TEAC) was found to be higher in the Racale extracts with a maximum value for Nicola and Sieglinde F cultivars (**Table 5**). A positive correlation, $R^2 = 0.8193$, was observed when the total TEAC activity and total phenol content of "early potatoes" in the extracts of both locations was considered. Interestingly, Nicola and Sieglinde F, which contain the highest carotenoid content (**Table 1**), also showed the highest antioxidant capacity, suggesting a synergistic radical scavenging activity between these lipophilic compounds and the other antioxidant components.

The ability of potato extracts (whole or peels) to scavenge single free radical species in biological systems has not previously been explored in detail. As these radical species have significant potential to damage biological tissues and the scavenging capacity of biological compounds may be considerably different, depending on the attacking oxidants, the characterization of the antioxidant capacity of "early potatoes" against three single radical scavengers was examined. The scavenging capacity was quantified toward peroxyl and hydroxyl radicals as well as against peroxynitrite by using the TOSC assay method (*31*). The methanol/water extracts affect the reaction of hydroxyl radical analysis by inhibiting the ethylene formation. For this reason the assay of hydroxyl radicals was performed by PBS-soluble extracts, while the assay of peroxyl radicals and peroxynitrite was performed by using methanol/water extracts.

The radical scavenging activity of all the freeze-dried extracts at varying volumes showed that the scavenging effect was enhanced with the increasing antioxidant amount to some extent, and then leveled out with subsequent lower increase (**Figure 1**). In **Figure 2**, the concentrations of the different potato extracts calculated at the corresponding TOSC values of 20%, 50%, and 80% confirmed the nonlinearity between antioxidant concentrations and antioxidant capacity. The results indicated that 6.0−10 μM gallic acid equivalents of methanol/water extracts, depending on the cultivar, are needed to inhibit the reaction against peroxyl radical for a TOSC value of 50% (**Figure 2a**), while a lower concentration of antioxidants (5.0−8.0 μM) is sufficient to obtain the same scavenge rate for peroxynitrite (**Figure 2b**). The highest antioxidant capacity against peroxyl radicals and peroxynitrite was found in the Nicola extract, closely followed by the Seiglinde F. For hydroxyl radicals, the antioxidant capacity of PBS extracts to obtain a 50% TOSC value was from 5.0 to 6.0 μM equivalents of gallic acid: the Seiglinde F required the lowest antioxidant concentration (**Figure 2c**). A similar capacity was obtained analyzing the extracts from potato cultivars grown in Monteroni.

The specific total oxyradical scavenging capacity, calculated by experimental TOSC value/potato dry weight, in comparison to peroxynitrite, hydroxyl radicals, and peroxyl radicals is shown in **Table 6**. The comparison between the results of peel extracts and whole tuber extracts leads to the conclusion that the antioxidant capacity of peels in "early potato" is 2−4 times higher than that in the whole tuber. It can also be observed that all peel and whole extracts showed a significant antiperoxyl capacity ($P < 0.05$), with some difference in scavenging capability either between the two groups (corresponding to the growing locations) or among the cultivars in each group (**Table 6**). In addition, the specific total oxyradical scavenging capacity against peroxynitrite evidenced a high scavenging capacity, which proved to be much higher in peel extracts from the Racale cultivated group (**Table 6**). The hydroxyl radical scavenging

Table 6. Specific Total Oxyradical Scavenging Capacity of Different "Early Potato" Extracts (Whole and Peel) against Peroxyl Radicals, Peroxynitrite, and Hydroxyl Radicals[a]

		scavenging capacity (TOSC unit mg^{-1} dw)					
		peroxyl radicals		peroxynitrite		hydroxyl radicals	
cultivar	field	whole	peel	whole	Peel	whole	peel
Nicola	Monteroni	92 ± 3 b	337 ± 18 f	99 ± 4 c	283 ± 15 ef	94 ± 6 b	147 ± 4 a
Sieglinde F	Monteroni	91 ± 4 a	273 ± 15 c	67 ± 3 a	233 ± 23 c	69 ± 1 a	152 ± 9 a
Isci 4052	Monteroni	81 ± 8 a	304 ± 5 d	62 ± 3 a	179 ± 2 b	78 ± 2 a	159 ± 2 b
Isci 67	Monteroni	94 ± 3 b	293 ± 12 d	58 ± 1 a	146 ± 4 a	61 ± 5 a	180 ± 3 c
Nicola	Racale	117 ± 2 d	417 ± 16 h	96 ± 5 c	321 ± 12 g	119 ± 4 c	203 ± 4 d
Sieglinde F	Racale	107 ± 5 c	256 ± 12 b	80 ± 5 b	259 ± 20 d	77 ± 9 a	169 ± 14 bc
Isci 4052	Racale	103 ± 1 c	121 ± 12 a	64 ± 1 a	299 ± 219 f	88 ± 6 b	168 ± 2 b
Isci 67	Racale	88 ± 3 a	316 ± 5 de	63 ± 2 a	301 ± 14 f	70 ± 3 a	163 ± 13 b

[a] Mean ± SD ($n = 6$). Mean values within a column with different letters are significantly different at $P < 0.05$.

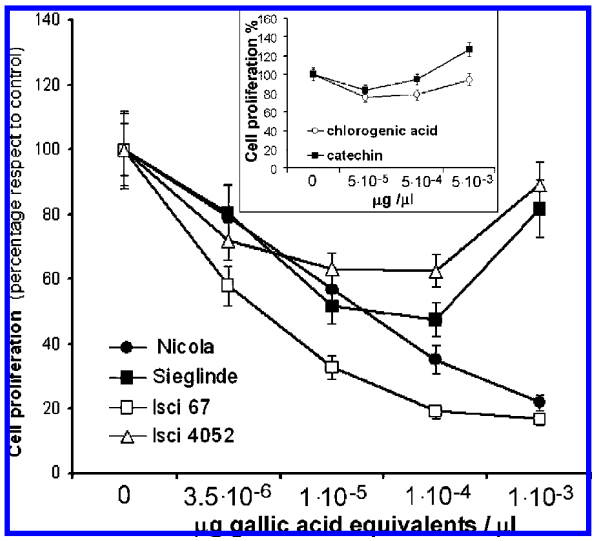

Figure 3. Effects of potato methanol/water extracts on cell proliferation in MCF-7. Mean ± SD, $n = 6$.

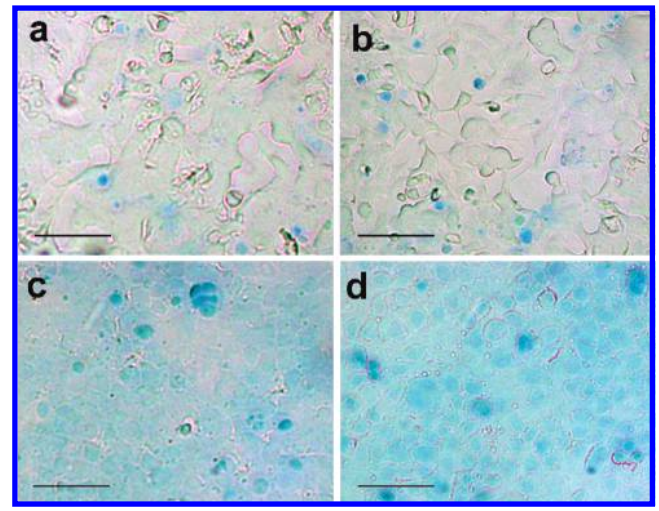

Figure 4. Viability of cells treated with potato phenol extract (cultivar Nicola): (**a**) solvent-treated control cells, (**b**) cells treated with potato extract containing 3.5×10^{-6} μg gallic acid equivalents/μL, (**c**) 1×10^{-5} μg gallic acid equivalents/μL, and (**d**) 1×10^{-3} μg gallic acid equivalents/μL.

activities were also found to show a noteworthy scavenging capacity in both Racale and Monteroni grown potatoes (**Table 6**). A highly significant linear correlation ($R^2 = 0.9613$) between total antioxidant capacity (as the sum of peroxyl radicals + peroxynitrite) and total phenol content of methanol extracts was established. Such high R^2 value suggested that the TOSC radical scavenging activity is related to the Folin−Ciocalteu assayed phenols and confirmed that the lipophilic phenols in the "early potatoes" were mostly responsible for antioxidant activity. A high correlation ($R^2 = 0.9613$) was also observed between Folin−Ciocalteu assayed phenols and TOSC activity against hydroxyl radicals.

Effect of Potato Extracts on MCF-7 Cell Growth and Cell Viability. The "early potato" extracts inhibited the breast cancer MCF-7 cell proliferation in a dose-dependent manner. Cell proliferation was determined 24 h after incubation in potato extracts by the MTS-based colorimetric assay. All "early potato" extracts were effective in inhibiting MCF-7 cell proliferation. Isci 67 and Seiglinde F extracts showed an inhibiting ability at concentration between 3.5×10^{-6} and 1×10^{-4} μg gallic acid equivalents μL^{-1}, the inhibitory effect decreasing up to these concentrations. Nicola and Isci 67 extracts exhibited a relatively strong inhibitory activity on MCF-7 cell proliferation at concentrations between 3.5×10^{-6} and 1×10^{-3} μg gallic acid equivalents μL^{-1} and their antiproliferative activities were higher than those of Isci 67 and Seiglinde F (**Figure 3**). Nevertheless, it is worth pointing out that in experiments performed by using potato Nicola and Isci 67 extracts at concentrations over 1×10^{-3} μg gallic acid equivalents μL^{-1}, the inhibitory effect on cell proliferation diminished and the number of cells increased (data not shown). Similarly, MCF-7 cell proliferation was inhibited by standard chlorogenic acid at concentrations between 5×10^{-5} and 5×10^{-3} μg μL^{-1} and by catechin at 5×10^{-5} and 5×10^{-4} μg μL^{-1}, but at higher concentrations an enhancement of cell proliferation was observed (**Figure 3**, insert).

The Nicola extract, which revealed the highest antiproliferative activity, was chosen for testing the morphological changes of MCF-7 cells induced by different concentrations of the extract (**Figure 4a−d**). MCF-7 cell incubated with potato extract containing 3.5×10^{-6} μg gallic acid equivalents μL^{-1} showed no morphological changes compared to control cells, whereas cells incubated with potato extract containing 10^{-5} μg gallic acid equivalents μL^{-1} displayed significant changes such as round shape and blunt outline (**Figure 4b**). These morphological alterations were even more pronounced with extract at 10^{-3} μg gallic acid equivalents μL^{-1} (**Figure 4d**). There was no significant difference in cell viability between cells treated with 3.5×10^{-6} μg gallic acid equivalents μL^{-1} and untreated cells (Trypan blue stained cells), while an increased number of dead cells induced by 10^{-5} and 10^{-3} μg gallic acid equivalents μL^{-1} of potato extract was observed (**Figure 5**). These results are consistent with those of recent studies indicating that antioxidants from fruits or vegetables (*32, 33*) can modify the cell cycle

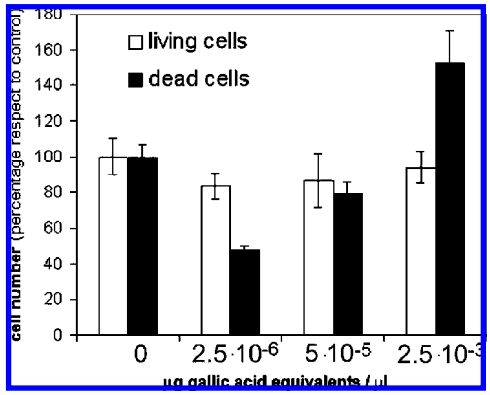

Figure 5. Quantitative analysis of viability in treated and untreated cell of different "early potato" extracts. Mean ± SD, $n = 6$.

in human breast cancer cell line MCF-7 and in several cancer cell lines (*34*). Resveratrol treatment of MCF-7 cells caused, at low concentrations, the inhibition of cell growth and cell accumulation at S phase transition of the cell cycle, accumulation not observed when cells were treated with high resveratrol concentrations (*35*). Raspberry extracts enriched in polyphenols have also been reported to reduce proliferation in a dose-dependent manner in human cervical cancer (HeLa) cells in vitro (*36*) and Nifli et al. (*37*) observed an arrest at phase S of the cell cycle in breast cancer cells T47D. Many other studies have confirmed that plant antioxidants, including ascorbic acid, carotenoids, and phenols (*38*) such as anthocyanins or anthocyanidins (*39*) and ellagic acid (*36, 40*), inhibit cancer proliferation in vitro. Depending on the experimental conditions, phenolic compounds can function as either antioxidants or prooxidants (*35*). However, the precise mechanisms of the anticancer effects of phenols remain largely unknown. The observed lack of antiproliferative effect at high concentrations of "early potato" extracts and chlorogenic acid and catechin standards could be explained taking into account that the concentrations used were likely out of the physiological range (*41*). In our experiments, extracts at low concentrations (assumed to be physiological concentration) showed a cytostatic effect with a decline of cell proliferation (**Figure 3**). In addition, Nicola potato extract at higher concentrations showed a cytotoxic effect and an increased number of dead cells (**Figure 4c,d**).

The data presented here indicate that there are differences in the in vitro inhibition of cancer cell proliferation by each potato extract tested, but a relationship between antiproliferative activity and phenol equivalents present in the extracts was only found for Nicola and ISCI 67 cultivars. This result cannot be explained by the presence in the potato extracts of methanol/water extractable compounds alone and this suggests that other phytochemicals may play a role in the antiproliferative activity of "early potatoes". The differences in cell proliferation inhibition induced by different potato extracts may be explained by the complex of antioxidants, which work additively or synergistically, accounting for the total activity.

In conclusion, the experimental data presented in this paper show that the large variations in total antioxidant capacity and total polyphenol, ascorbic acid, and carotenoid content of the "early potato" were due to documented factors such as cultivars, soil, and other environmental factors. Comparison of the results obtained from the two growing areas showed that in "early potato" cultivars the main bioactive compounds in both cultural systems were high and variable between crops and within the genotype studied. The differences in specific antioxidant capacity observed across sites suggest that potato harvest location (environmental conditions) influenced the accumulation of antioxidant compounds. The characteristic of the Racale soil (the Racale soil is a red silt-sand soil, less compacted, well-drained, and well-aerated than that in Monteroni) together with a relatively low incidence of the winter−spring temperatures could be possible reasons for the significant influence on the vegetative growth of potato crops and the level of bioactive compounds.

These results highlight the importance of phenol compounds in the antioxidant behavior of potato extracts and also indicate that the phenol compounds significantly contribute to the total antioxidant capacity. The results are in general agreement with previous reports (*18, 19*), suggesting that many of these hydrophilic (*27*) and lipophilic (*29, 32, 42*) phenolics present in potatoes have antioxidant capacity and may protect cells against the oxidative damage (*31*). It is known that oxidative damage induces increase of cancer risk and it is estimated that one-third of cancer deaths could be avoided through an appropriate intake of phenols or other antioxidants based on daily fruit and vegetable consumption (*43*). Chun et al. (*43*), analyzing the daily consumption of phenolics and total antioxidant capacity from fruit and vegetables, estimated that daily intake of phenolics, flavonoids, and antioxidants was 450 mg gallic acid equivalent (GAE) and that, in relation to the recommended dietary intakes (171.4 g day^{-1} $person^{-1}$ of potato), potatoes contributed 60.5 mg of GAE, equivalent to 40% of total antioxidants. On the basis of the same consumption of potato per day, the contribution to daily intake in total phenols for the "early potatoes" was high, especially for Nicola cultivar (Racale site), and accounted for 75 mg GAE 100 g^{-1}.

Alleged health-promoting effects of phenols are usually attributed to their powerful antioxidant activities and are thought to act as prooxidant by scavenging reactive nitrogen, chlorine, and oxygen species (*44*). Extracts of "early potato" effectively scavenged in vitro hydroxyl radicals, or peroxynitrite and peroxyl radicals in a concentration-dependent manner and these data are in agreement with previous reports that indicated antioxidant compounds can effectively scavenge various reactive oxygen species/free radicals under in vitro conditions (*27, 29, 36*). The activity of antioxidant molecules is nearly proportional to the number of OH present in their molecular structure (*45*). In "early potato" extracts, catechin and clorogenic acid constituted 90% of total phenols and, considering that the two chemical species both contain five OH groups (three of which are reported to be very reactive), the contribution of these compounds to the total antioxidant activity could be relevant (*45*). Moreover, "early potatoes" have a relatively high content of ascorbic acid and an amount of carotenoids, so the contribution of these compounds to total antioxidant activity can also be considerable.

In summary, the results presented in this study clearly demonstrate that the "early potatoes" contain an elevated amount of several antioxidant compounds with considerable antioxidant activity. The antioxidant values of these tuber crops are higher or comparable to known sources of other cultivated potato varieties, indicating that these crops have the potential to be considered as important novel sources of nutraceuticals. The characterization of the phytochemicals will be used to estimate the contribution of "early potato" to antioxidant intake of the consumers. Furthermore, scientists can use the data to increase antioxidant and other phythochemical components in foods or in breeding programs to improve the nutritive status of this crop.

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