JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Cooking on the Anthocyanins, Phenolic Acids, Glycoalkaloids, and Resistant Starch Content in Two Pigmented Cultivars of *Solanum tuberosum* L.

NADIA MULINACCI,*,† FRANCESCA IERI,† CATIA GIACCHERINI,† MARZIA INNOCENTI,† LUISA ANDRENELLI,‡ GIULIA CANOVA,§ MARCO SARACCHI,|| AND MARIA CRISTINA CASIRAGHI§

Department of Pharmaceutical Science, University of Florence, via Ugo Schiff 6, 50019 Sesto Fiorentino (FI), Italy, Department of Agronomy and Land Management (DiSAT), University of Florence, P.zale delle Cascine 18, 50144 Firenze, Italy, and DiSTAM-sezione Nutrizione, and Istituto di Patologia Vegetale, University of Milan, Via Celoria 2, 20133 Milan, Italy

HPLC/DAD/MS analysis of the phenolic acids and anthocyanin content of three cultivars of *Solanum tuberosum* L. (Vitelotte Noire, Highland Burgundy Red, with pigmented flesh, and Kennebec with white pulp) was performed. The analyses were carried out both on fresh tubers and after cooking treatments (boiling and microwaves). Starch digestibility and the % of resistant starch were also determined on cooked tubers by in vitro methods. For the pigmented potatoes, the heating treatment did not cause any changes in the phenolic acids content, while anthocyanins showed only a small decrement (16−29%). The cv. Highland Burgundy Red showed anthocyanins and phenolic acid concentrations close to 1 g/kg and more than 1.1 g/kg, respectively. Vitellotte Noire showed the highest amounts of resistant starch. Potato starch digestibility and % of resistant starch, considered as a component of dietary fiber, were affected both by cultivar and by heating/cooling treatments.

KEYWORDS: Vitelotte Noire; Highland Burgundy Red; anthocyanins; starch digestibility; resistant starch; microwaves; boiling

INTRODUCTION

Potato, together with common cereals, is one of the most important food crops worldwide. In Europe, per capita intake of potato has been estimated to range from 40 kg/year for Italy and France to 102 kg/year for Greece (data from INEA 2006). Among the minor constituents of potato, chlorogenic acid and its isomers (*1*) are well known. These compounds, together with the phenoloxidases, are involved in the enzymatic browning process of the tuber (*2*) but are also well recognized as natural antioxidants.

Colored tubers, even if not commonly found in our habitual diet, are now available on the European and Italian markets, and a potentially large number of consumers can acquire these products. However, in some regions of South America, typical *Solanum tuberosum* varieties with intense blue-red colored pulp are well known. Yet scant data are available on the composition of these ancient varieties (*3*), and on their anthocyanins content, the pigments responsible for the particular color of tubers. Their presence in the plants influences the self-protection against biotic and abiotic stress and can contribute to chemotaxonomic characterization. In the past decade, great interest has developed regarding evaluation of the anthocyanin content in the human diet, due to the potential health benefits of these compounds (*4*). It has been demonstrated that these pigments are rapidly adsorbed at the stomach level (*5*) and were detectable in urine and plasma (*6*), where they protect LDL against oxidation (*7*). It is also well established that anthocyanins inhibit digestive enzyme activity, such as α-glucosidase, and they can reduce blood glucose levels after starch-rich meals (*8, 9*).

No data are currently available in the literature on the content of glycoalkaloids in blue-red potatoes previously studied (*10*). Nevertheless, their content must be evaluated to ensure the safety of the product for human consumption. In fact, a maximum admissible concentration of 200 mg/kg fresh tuber has been established as the upper safety level (*11*). Given that potato is not consumed fresh by humans, it is fundamental to measure the concentration of bioactive compounds such as phenolic acids, glycoalkaloids, and anthocyanins after cooking.

Recent advances in understanding the role of starches in health have underlined the importance of the rate and extent of starch digestion on postprandial glucose and insulin responses, metabolic parameters involved in the etiology of chronic

* Corresponding author. Phone: +390554573773. E-mail: nadia.mulinacci@unifi.it.
† Department of Pharmaceutical Science, University of Florence.
‡ Department of Agronomy and Land Management (DiSAT), University of Florence.
§ DiSTAM-sezione Nutrizione, University of Milan.
|| Istituto di Patologia Vegetale, University of Milan.

10.1021/jf801521e CCC: $40.75 © 2008 American Chemical Society
Published on Web 11/20/2008

diseases such as diabetes, obesity, and cardiovascular disease (*12*). Resistant starch (RS) has been defined as the sum of starch and starch-degradation products that reaches the human large intestine (*13*), and it is now regarded as a fraction of dietary fiber (*14*), with a positive impact on colonic welfare and lipid metabolism (*15*). Some authors established that the main RS sources in the Swedish diet are bread and potato products, which contribute approximately 1.3 and 1.2 g of RS per day, respectively (*16*); in Italy, estimated RS intakes from potatoes are lower, on average 650 mg per day, covering about 8−10% of the total RS intake in the Italian diet (*17*).

Cold storage of boiled potatoes generated appreciable amounts of RS, and it was shown that the high glycaemic and insulinaemic features commonly associated with potato meals were reduced by serving cold potato products (*18*).

In this context, the primary goal of the present study was to determine the potential health properties of two pigmented potatoes by measuring their content of phenolic acids, glycoalkaloids, and anthocyanins not only on the fresh tuber, but also after boiling and microwave cooking processes. As the main constituent of potatoes is starch, and because the nutritional quality of starch strongly depends on starch structure (often related to botanical origin, processing, and interaction with other components of vegetables), the second aim of the study was to study the starch fraction of these tubers with particular attention to its rate and the extent of digestion.

The selected cultivars were a blue-flesh potato from France, cv. Vitelotte Noire, and a red-flesh potato cultivated in Italy, cv. Highland Burgundy Red. The results were compared to those obtained from a common white pulp potato variety, cv. Kennebec.

EXPERIMENTAL PROCEDURES

Samples Extraction. The Vitelotte Noire tubers were purchased from Generalfrutta srl (Verona-Italy). Highland Burgundy Red and Kennebec varieties were kindly supplied by Professor Vincenzo Vecchio from the DiSAT of the University of Florence.

For each variety, the fresh potatoes (from 500 to 1000 g) were cut, treated with liquid nitrogen, and immediately ground. The obtained powder (30 g) was extracted three times with a total volume of 300 mL of 70% EtOH adjusted to pH 1.8 by HCOOH, over a 1 h period under stirring, and centrifuged by Hermle LaborTechnik (5000 rpm × 5 min). The three extracts were maintained separate and were analyzed as such by HPLC.

Cooking Processes. Three whole tubers of approximately similar size (about 100 g) were used, and the cooking times were chosen to obtain a palatable product. For the boiling process, potatoes were boiled for 20 min with 2.3 g of water for each gram of sample. The microwave treatment was carried out at 700 W for 8 min, using 0.1 g of water for each gram of sample.

After being cooked, the potatoes were cooled at room temperature and extracted in two steps with a hydro alcoholic mixture EtOH/H_2O acidified by formic acid (1.8% vv) 7:3, with a final ratio of 1 g/10 mL as described for the fresh potatoes. For every experiment, each of the three whole tubers was treated separately to obtain three extracts for HPLC/DAD/MS analysis.

HPLC/DAD/MS Analysis. Analysis was carried out using a HP-1100 liquid chromatograph equipped with a DAD detector and a HP 1100 MSD API-electrospray (Agilent-Technologies, Palo Alto, CA) operating in positive ionization mode under the following conditions: gas temperature 350 °C, nitrogen flow rate 10.0 L min^{-1}, nebulizer pressure 35 psi, quadrupole temperature 30 °C, capillary voltage 4000 V, and applied fragmentors in the range 50−250 V.

The column was a Synergi Max RP 80 A (4 μm; 150 mm × 3 mm i.d.) from Phenomenex. The mobile phase was (A) water pH 2.0 acidified by orthophosphoric acid (only for HPLC/DAD) or formic acid (for the HPLC/DAD/MS analysis) and (B) acetonitrile. The following multistep linear gradient was applied: from 95% to 78% of A in 8 min, 4 min to reach 74% A, then 13 min to arrive at 65% A, and finally 3 min to reach 100% B with a final plateau of 4 min. Total time of analysis was 32 min, flow rate was 0.4 mL/min, and oven temperature was 26 ± 0.5 °C.

Quantitative Determination of Phenolic Acids and Anthocyanins. The phenolic acids were evaluated by HPLC/DAD using a five-point calibration curve of chlorogenic acid (Extrasynthese-Genay Cedex-France) at 330 nm ($r^2 \geq 0.999$), while the anthocyanin content was determined by HPLC/DAD using a five-point calibration curve of malvin chloride (mw 691 from Extrasynthese-Genay Cedex-France) at 520 nm ($r^2 \geq 0.999$). All of the anthocyanins are expressed in malvine, also malvidin 3,5-*O*-diglucoside (mw 655).

The quantitative determination of glycoalkaloids was determined by HPLC/DAD at 208 nm using five-point calibration curves ($r^2 \geq 0.999$) of α-chaconine and α-solanine (Sigma Aldrich Laborchemikalien GmbH, Germany).

Microscopy Analysis. Samples were prepared using the freeze-drying technique described by Petrolini and colleagues (*19*). Cylinders (10 mm long and 3 mm in diameter) of raw and differently cooked potato flesh were cut off from different tubers of each potato cultivar.

The samples were packed singly in perforated aluminum foil to permit gaseous exchange. The specimens were frozen by immersion in liquid nitrogen. Tissues treated in this way turned crumbly, and it was possible to fracture them directly in the packets. Frozen samples were quickly transferred to the plate of a Pearse Tissue Dryer (mod. EPD3 Edwards High Vacuum, Ltd. U.K.) and maintained at −40 °C under vacuum for 12 h. Then samples were placed onto the microscope sample holder coated with a thin film of gold and examined under a Leo 438VP scanning electron microscope (SEM) (Leo Electron Microscopy, Cambridge, U.K.). Image analysis and measurements were assessed directly by means of the software that controls the electron microscope. The size of 400 starch granules for each potato cultivar was assessed.

Total (TS) and Resistant Starch (RS) Assessment. Total and resistant starch were evaluated on potato samples immediately after cooking (boiling or microwave) and after a 24 h cooling period at 4 °C.

TS and RS were determined using the enzymatic procedure proposed by Champ (*20*), slightly modified as follows. Aliquots of sample (about 100 mg of starch, estimated according to published values of starch content in white potatoes: http://www.ieo.it/bda2008/uk/SearchForName.aspx) were dispersed in 0.1 M, 4 mM $CaCl_2$, acetate buffer at pH 5.2, hydrolyzed for 16 h with pancreatic α-amylase (EC 3.2.1.1; Sigma cat A 3176), washed with ethanol (90% v/v), and the dry residues dispersed in 2 M KOH and hydrolyzed with amyloglucosidase (EC 3.2.1.3). Free glucose was then analyzed using a glucose analyzer (YSI 2300; Yellow Spring Instruments, Yellow Spring, OH) and reported in polymeric form (by a multiplicative factor of 0.9) to calculate TS and RS.

Starch Digestibility. Isoglucidic (about 2000 mg of starch) amounts of sample were digested in vitro as previously described (*21*). Briefly, after acidification to pH 2 with 8 M HCl, samples were digested for 1 h at 37 °C with hog pepsin (EC 3.4.23.1; Sigma cat P7012). At the end of pepsin digestion, to mimic intestinal starch digestion, mixtures were incubated with 50 UI of pancreatic α-amylase (EC 3.2.1.1; Sigma cat A 3176) in dialysis bags (cutoff 6000−8000 Da, Spectrapor, Spectrum Medical Industries) at 37 °C and pH 6.9, against 450 mL of 20 mM Na-phosphate buffer containing 10 mM NaCl. Every 30 min, for a 5 h period, 1 mL aliquots of dialysate were taken for total dialyzable reducing sugar analysis (*22*).

Differential Scanning Calorimetry (DSC). Fresh potato was cut into a central longitudinal slice, and cylinders (with a diameter of 3.5 mm punched from the selected tissue zones) were cut to approximately 1 mm thick slices. Samples were placed in weighed, coated aluminum pans, which were immediately hermetically sealed and accurately reweighed with a Mettler MX5 microbalance. No water was added, according to Karlsson and Eliasson (*23*). Analyses were performed on a Mettler TA4000 Star^e software apparatus (Mettler Toledo, Switzerland) equipped with a DSC 25 cell, over the temperature range of 30−105 °C with a scanning rate of 10 °C min^{-1}. The instrument was

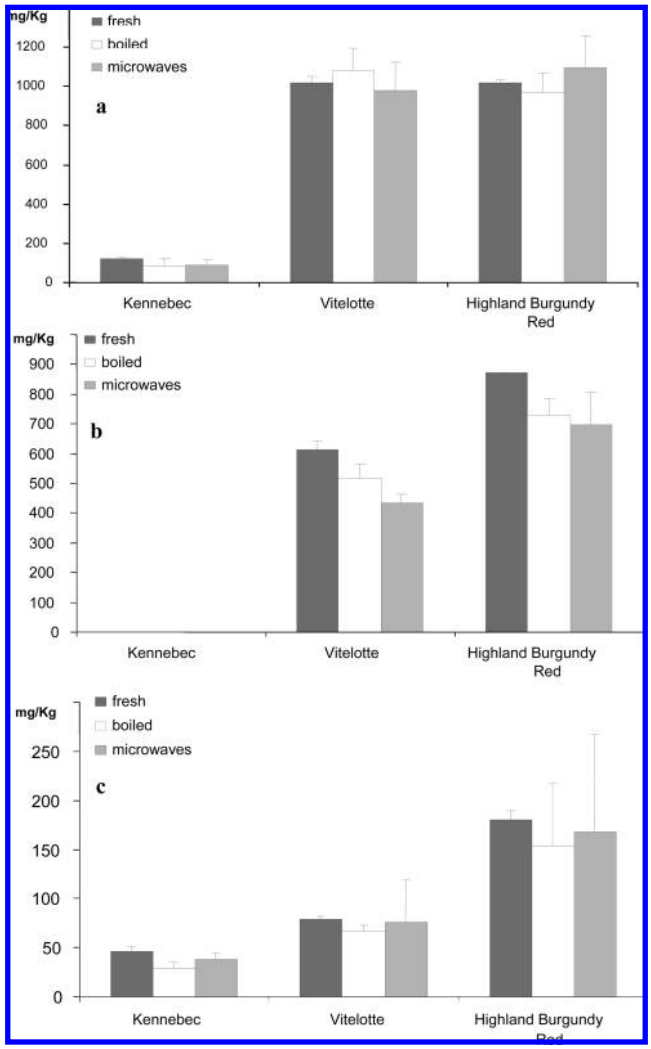


Figure 1. Amounts of phenolic acids, anthocyanins, and glycoalkaloids for cultivars Vitelotte Noire, Highland Burgundy Red, and Kennebec. Each datum is expressed as mg/kg of fresh weight, and it is a mean of three different determinations, each on a whole tuber: (**a**) phenolic acids, expressed as chlorogenic acid at 330 nm; (**b**) anthocyanins, expressed as malvin at 520 nm; (**c**) glycoalkaloids, calculated as the sum of α-chaconine and α-solanine at 208 nm.

calibrated using indium as standard (99.98% purity; melting point 156.61 °C; fusion enthalpy 28.71 J/g), and an empty pan was used as reference. After the DSC scan, the dry matter content was determined by puncturing the pans and drying them in an oven at 105 °C until constant weight. Transition enthalpy (ΔH expressed as J/g dry matter), onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) were measured. The experimental values are the means of six different measurements, obtained from three different tubers for each variety.

RESULTS AND DISCUSSION

Phenolic Acids, Anthocyanins, and Glycoalkaloids. The phenolic acid profile of the flesh (pulp) is not peculiar to the variety: the main metabolite was shown to be chlorogenic acid, with minor amounts of neochlorogenic and ferulic acids for all of the considered samples. As shown in **Figure 1a**, the total amount of these secondary metabolites in the pigmented cultivars (mean range 1018−1016 mg/kg) is notably higher in comparison to the content observed in the white-flesh variety, Kennebec (121 mg/kg). Different from literature data that indicate a loss (up to 70−80%) of phenolic acids after boiling of cut potatoes (*24*), in our experiments the content of these molecules in the whole tuber remained unmodified after the two heating treatments. This disagreement may be related to several factors depending on the use of whole unpeeled tuber. The reduction in loss of phenols can be related to the browning phenomenon during the cut, to a reduced pulp surface exposed to hot water, and also to the presence of peel.

The anthocyanin profile was determined for both pigmented tubers, and the chemical structures of all of the molecules were tentatively determined by their UV−vis and mass spectra in positive ionization mode at different fragmentation energy and by comparison with literature data (*10, 25*). The UV−vis spectra were diagnostic of the anthocyanidin structure, which was confirmed by the fragmentation pattern in the mass spectra. These latter findings indicate the molecular weight and also the number of glycosides together with the presence of acylated derivatives (**Table 1**). For the cv. Vitelotte Noire, the profile in **Figure 2** shows the 3-*O*-*p*-coumaroyl rutinoside-5-*O*-glucosides both of malvidin and of petunidin as major constituents, together with cynnamoyl rutinosil glucosides of malvidin, petunidin, and delphinidin. These minor constituents (compounds 2, 3, 6, 8) were not detected in a previous work on this cv. (*10*) that highlighted only the presence of the major anthocyanins (compounds 1, 4, and 7). The analysis of more than three different commercial samples of Vitelotte Noire purchased on the Italian market in different seasons and years confirmed this peculiar profile of the pigmented fraction.

With regard to the Highland Burgundy Red variety, the anthocyanin profile of the pulp (**Figure 3**) confirmed the presence of pelargonidin 3-*O*-rutinoside 5-*O*-glucoside and of pelargonidin 3-*O*-*p*-coumaroylrutinoside 5-*O*-glucoside according to previous results (*10*). Furthermore, also pelargonidin 3-*O*-feruloyl rutinoside 5-*O*-glucoside and peonidin glycosides have been highlighted as minor constituents.

From a quantitative point of view, the fresh tuber of these colored cultivars shows relatively high levels of anthocyanis (expressed as malvine), ranging from 613 to 871 mg/kg, corresponding to 123 or 174 mg for a portion (200 g row) of Vitelotte Noire and of Highland Burgundy Red, respectively.

The HPLC/DAD profiles after cooking were the same as those obtained for the respective uncooked potatoes, with the ratios among the area values of the different pigments within each sample remaining unaltered. Nevertheless, a decrease of the total anthocyanin content, easily observed from the purple-violet color of the boiling water, was highlighted for both of the red varieties. Unexpectedly, given that their stability is strongly dependent on the pH value in water media and also on the temperature, the % loss of these pigments, with respect to the fresh samples, was relatively low (range 16−29%) (**Figure 1b**). In particular, due to the partial loss of water in the cooked tuber, for the processed tubers of Highland Burgundy Red the anthocyanins and the phenolic acid amounts arrived close to 1 g/kg and more than 1.1 g/kg, respectively.

After the samples were cooked, no significant variations were observed for the glycoalkaloid content of all of the samples, even if a greater variability of results (**Figure 1c**) was observed for the pigmented varieties, especially for those treated with microwaves.

In any case, it can be underlined that all of the mean values remained below the maximum level of 200 mg/kg fixed for the commercialization of fresh tuber (*11*), while, to the best of the authors' knowledge, a maximum level for the cooked tubers does not exist.

Nowadays, even if only partial information is available on the quantities of polyphenols that are consumed daily throughout

Table 1. MS Data Obtained after Positive Ionization of the Anthocyanin Extracts Applying Various Fragmentation Energies (from 120 to 250 V)[a]

peak[b]	proposed anthocyanins[c]	molecular ion [M^+] (*m*/*z*)	fragment ions (*m*/*z*)
Vitelotte Noire			
1	mal 3-*O*-rut-5-*O*-glu	801	**331**, 493, 639
2	pet 3-*O*-caf-rut-5-*O*-glu	949	**317**, 479, 787
3	delp 3-*O*-*p*-coum-rut-5-*O*-glu	919	**303,** 465, 757, 773
4	malv 3-*O*-caf-rut-5-O-glu	963	**331**, 493, 801
5	pet 3-*O*-*p*-coum-rut-5-*O*-glu	933	**317**, 479, 771, 787
6	pet 3-*O*-ferul-rut-5-*O*-glu	963	**317**, 479, 787, 801
7	mal 3-*O*-*p*-coum-rut-5-*O*-glu	947	**331**, 493, 785, 801
8	mal 3-*O*-ferul-rut-5-*O*-glu	977	**331**, 493, 801, 815
Highland Burgundy Red			
9	pel 3-*O*-rut-5-*O*-glu	741	**271**, 433, 579
10	pel 3-*O*-rut	579	**271**
11	pel derivative		**271**, 488
12	pel 3-*O*-caf-rut-5-*O*-glu	903	**271**, 433, 741
13	pel 3-*O*-cis-*p*-coum-rut-5-*O*-glu	887	**271**, 433, 725
14	pel derivative		**271**, 293, 695
15	pel 3-*O*-*p*-coum-rut-5-*O*-glu and	887	**271**, 433, 725, 741
	peon 3-*O*-*p*-coum-rut-5-*O*-glu	917	**301**, 463, 755, 771
16	pel 3-*O*-ferul-rut-5-*O*-glu and	917	**271**, 433, 741, 755
	peon 3-*O*-ferul-rut-5-*O*-glu	947	**301**, 463, 771, 785
17	pel 3-*O*-*p*-coum-rut	725	**271**
18	pel 3-*O*-ferul-rut	755	**271**

[a] The aglycone ions are indicated in boldface. [b] Peak numbers refer to **Figures 1** and **2**. [c] Abbreviations used: pel, pelargonidin; peo, peonidin; pet, petunidin; mal, malvidin; delf, delfinidin; rut, rutinoside; glu, glucoside; ferul, feruloyl; *p*-coum, *para*-coumaroyl; caff, caffeoyl.

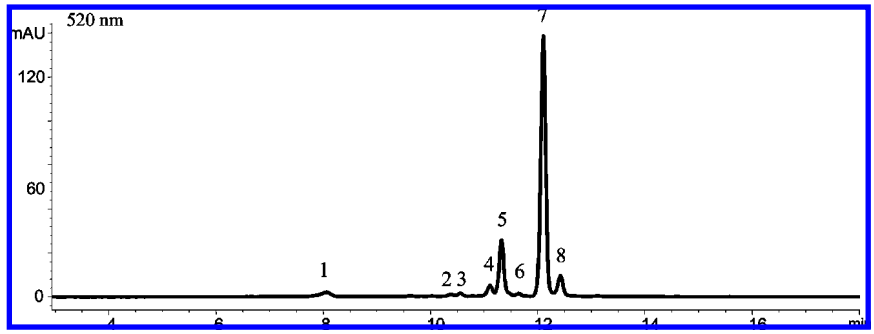


Figure 2. HPLC profile at 520 nm of Vitelotte Noire extract. The following molecules were tentatively identified: 1, malvidin 3-*O*-rutinoside-5-*O*-glucoside; 2, petunidin 3-*O*-caffeoyl-rutinoside-5-*O*-glucoside; 3, delphinidin 3-*O*-*p*-coumaroyl-rutinoside-5-*O*-glucoside; 4, malvidin 3-*O*-caffeoyl-rutinoside-5-*O*-glucoside; 5, petunidin 3-*O*-*p*-coumaroyl-rutinoside-5-*O*-glucoside; 6, petunidin 3-*O*-feruloyl-rutinoside-5-*O*-glucoside; 7, malvidin 3-*O*-*p*-coumaroyl-rutinoside-5-*O*-glucoside; 8, malvidin 3-*O*-feruloyl-rutinoside-5-*O*-glucoside.

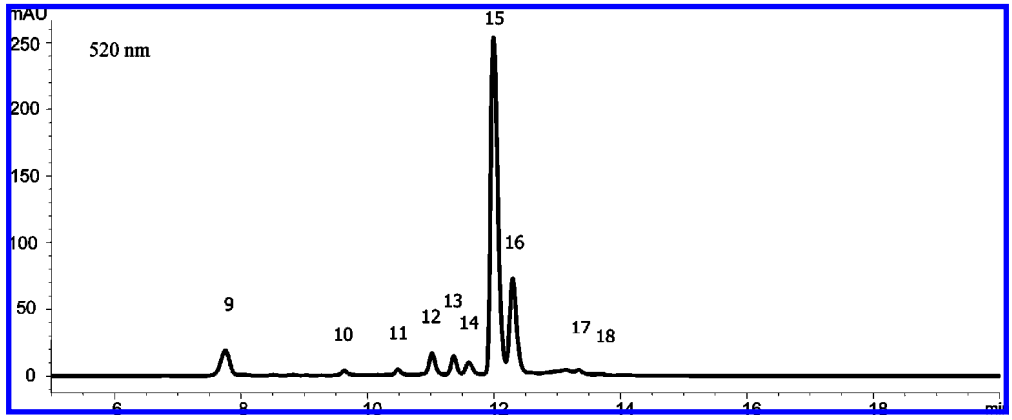


Figure 3. HPLC profile at 520 nm of Highland Burgundy Red extract. The following molecules were tentatively identified: 9, pelargonidin 3-*O*-rutinoside-5-*O*-glucoside; 10, pelargonidin 3-*O*-rutinoside; 11, pelargonidin derivative; 12, pelargonidin 3-*O*-caffeoyl-rutinoside-5-*O*-glucoside; 13, pelargon+idin 3-*O*-cis-*p*-coumaroyl-rutinoside-5-*O*-glucoside; 14, pelargonidin derivative (*m*/*z* 695); 15, pelargonidin 3-*O*-*p*-coumaroyl-rutinoside-5-*O*-glucoside and peonidin 3-*O*-*p*-coumaroyl-rutinoside-5-*O*-glucoside; 16, pelargonidin 3-*O*-feruloyl rutinoside-5-*O*-glucoside and peonidin 3-*O*-feruloyl-rutinoside-5-*O*-glucoside; 17, pelargonidin 3-*O*-*p*-coumaroyl-rutinoside; 18, pelargonidin 3-*O*-feruloyl-rutinoside.

the world, some authors (*26*) suggest that about 1 g/die can be considered as a reference value for dietary flavonoid intake. Thus, a portion (about 200 g row) of these colorful potato cultivars could consistently contribute to the daily intakes of these functional compounds.

Microscopy Observations. SEM images (**Figures 4** and **5**) show differences in tuber microstructure of raw and differently cooked potatoes. Slightly covered by cellular material, starch granules from the three different cultivars show similar shape but different size distributions (**Figure 4**). Starch granules in

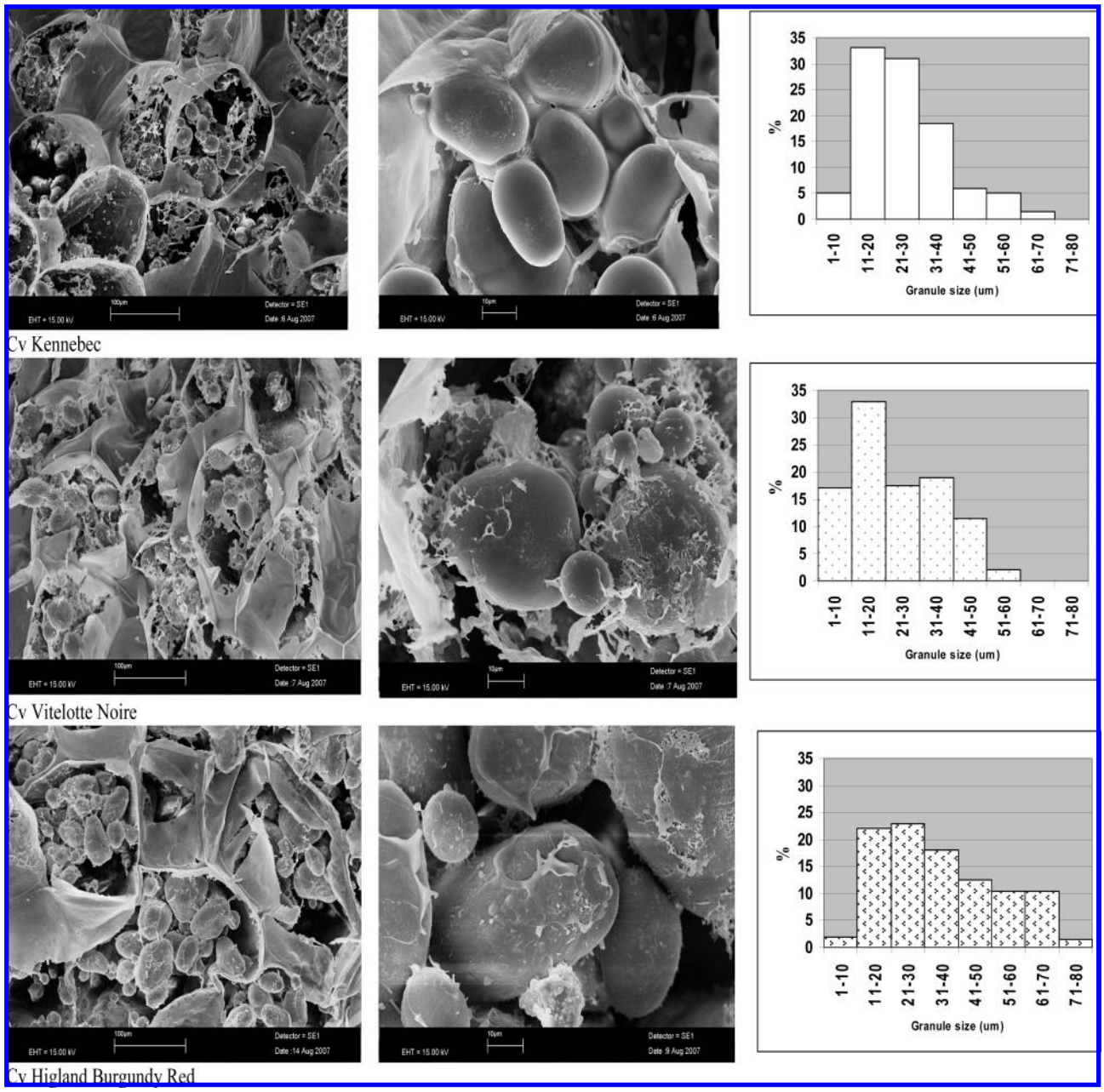


Figure 4. SEM micrograph (×1000) and size distribution range, expressed as percentage of starch granules within the indicated range size on total counted granules ($n = 400$), in raw samples of the three potato varieties.

cv. Kennebec appear to have a narrower range of size distribution, with most granules (about 75%) falling within a range of 11−40 μm. In contrast, the size of granules ranged widely in both cv. Vitelotte Noire and Highland Burgundy Red, showing a rather flattened size distribution, with higher percentages of large granules in particular in cv. Highland Burgundy Red. These data are in line with the wide range of potato starch granule size (10−80 μm) evidenced also by other authors for sweet potatoes (*27*).

Cooking significantly affected the microstructure of potatoes. Upon water absorption and heating, starch granules swell, resulting in different changes in microstructure in relation to potato cultivar and cooking method (**Figure 5**). In boiled cv.'s Kennebec and Highland Burgundy Red, a porous sponge-like structure is evident, probably promoted by the solubilization of amylose and amylopectin fractions during boiling. In boiled cv. Vitelotte Noire, however, a less porous network of starch gel is present, which is very similar to that induced by microwave cooking on cv. Kennebec (**Figure 5**). As suggested by Błaszczak et al. (*28*), microwave heating probably affected cellular water evaporation, thus limiting starch gelatinization and solubilization.

Differential Scanning Calorimetry (DSC). The thermal properties of starch in fresh potato samples were analyzed by DSC, and the results are presented in **Figure 6**. Cultivar was found to have only a slight effect on both the values of T_0, T_p, and T_c, thus suggesting only minor differences in gelatinization properties of the starch granules in the tested varieties. Onset and peak temperatures of colored potatoes resulted about 1−2 °C higher than those observed in the white-flesh sample, while enthalpy values, evaluated in cv. Kennebec, were higher than those of the cv.'s Vitelotte Noire and Highland Burgundy Red.

Starch Digestibility. Starch availability to digestive enzymes plays a key role in the rate of starch digestion and thus is considered a predictive parameter of food glycemic impact (*21*).

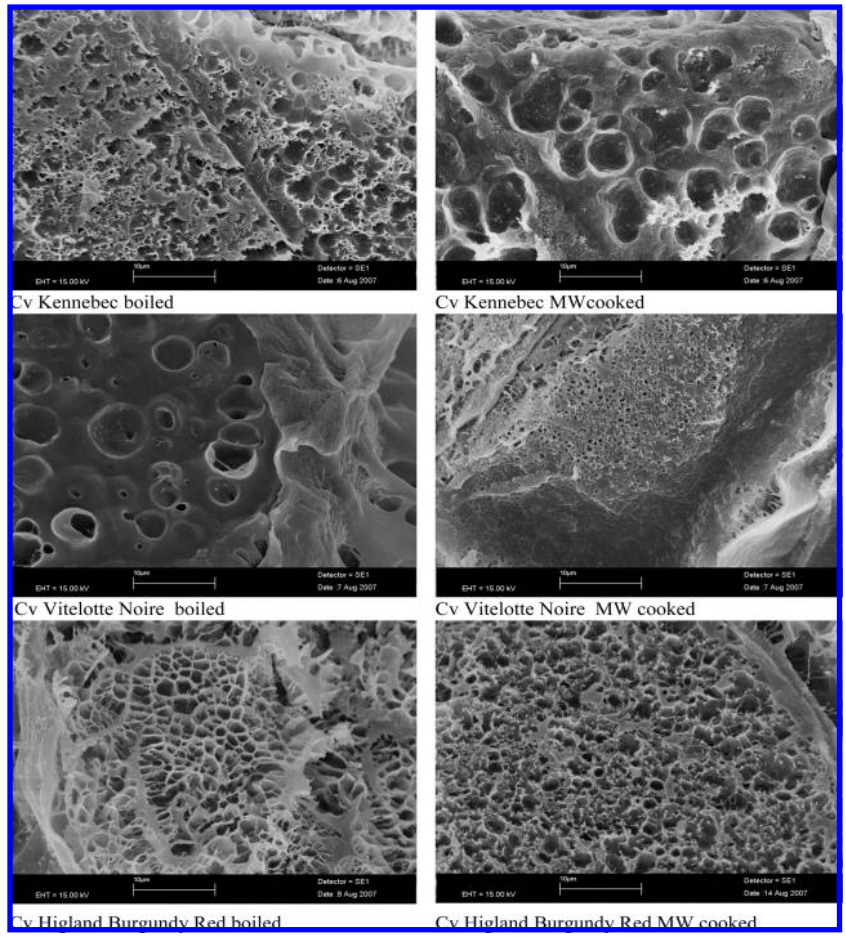


Figure 5. SEM micrograph (×2000) of boiled and microwave-cooked starch granules in samples of the three potato varieties.

Table 2. Total and Resistant Starch Values of the Three Potato Varieties[a]

	boiled			MW cooked		
	water %	TS %	RS %	water %	TS %	RS %
Kennebec	74.20 ± 0.86	16.62 ± 1.35	2.04 ± 0.67	57.58 ± 0.91	32.14 ± 4.37	2.68 ± 0.35
Vitelotte Noire	72.71 ± 1.36	21.89 ± 4.26	4.18 ± 0.47	50.43 ± 1.44	26.57 ± 2.04	6.31 ± 0.40
Highland Burgundy Red	77.25 ± 0.27	14.72 ± 1.28	1.76 ± 0.28	69.30 ± 0.08	19.61 ± 0.88	2.85 ± 0.12

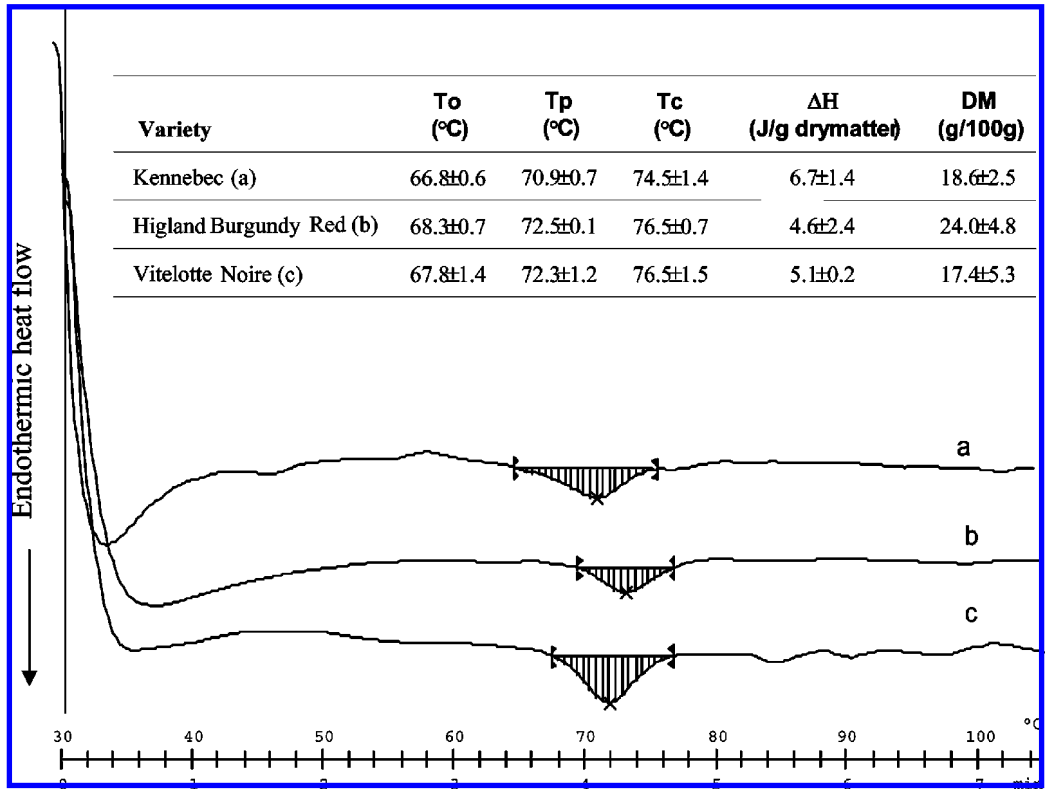
[a] Values are the mean ± standard deviations ($n = 4$). TS = total starch; RS = resistant starch.

In this study, some differences in the digestibility of starch were observed in relation to both the cultivar and the cooking methods. **Figure 7** reports the starch hydrolysis curves of boiled and microwaved samples of the three potato varieties. As expected, microwave cooking tends to reduce starch availability to digestive enzymes, as shown by hydrolysis curves constantly lower than those obtained for boiled potatoes.

Some authors (*29*) demonstrated that this behavior can be related to the fact that the crystallinity of potato starch increases during microwave irradiation, while conventional boiling tends to destroy the crystalline structure. Moreover, microwave treatment caused more advanced changes in the microstructure of tubers, which appears more dense and packed especially for the cv. Vitelotte Noire (**Figure 5**), and, in turn, it may result in a limited starch availability for digestion.

Starch digestibility seems also to be related to the cultivar: colored varieties, and in particular the cv. Highland Burgundy Red, showed a higher rate of starch digestibility in comparison with the white cultivar. This observation appears in contrast with recent findings by McDougal et al., which emphasize the role of some polyphenols as negative effectors of starch digestion (*8, 9*). These authors demonstrated that several polyphenolic components of fruit may influence different steps in starch digestion and in particular that anthocyanins are most effective in α-glucosidase inhibition, while some soluble tannins are mainly involved in modulating α-amylase activity. It must be underlined that the in vitro procedure used in the present study took into account only the α-amylase enzymatic activity. Moreover, the pigmented potatoes that we tested do not contain the tannins. Thus, it is likely that in our study the rate of starch digestibility, mainly assessed on the basis on α-amylase digestion kinetics, is not affected by the anthocyanin fraction of colored potatoes. In addition, our results were obtained on an actual vegetal sample in which the molar ratio between starch and polyphenolic compounds (that can act as enzyme inhibitors) may be different from the samples used in the cited work.

Enzymatic hydrolysis of native starch granules is affected by different parameters such as granule structure, crystal type, granule size, amylose and amylopectin ratio, average molecular weight, and the presence of lipids and proteins (*30*). The effect of particle size on enzymatic hydrolysis of native starch and starchy foods is expressed in terms of available surface area per mass for enzymatic action both in vivo and in vitro. From a kinetics point of view, a relationship between enzymatic



Variety	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g drymatter)	DM (g/100g)
Kennebec (a)	66.8±0.6	70.9±0.7	74.5±1.4	6.7±1.4	18.6±2.5
Higland Burgundy Red (b)	68.3±0.7	72.5±0.1	76.5±0.7	4.6±2.4	24.0±4.8
Vitelotte Noire (c)	67.8±1.4	72.3±1.2	76.5±1.5	5.1±0.2	17.4±5.3

Figure 6. DSC thermograms of in situ starch evaluation and relative measured parameters for the three varieties. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), transition enthalpies (ΔH, J/g dry matter), and dry matter contents (DM, g/100 g). Values are the mean of six determinations (from three tubers) ± standard deviations.

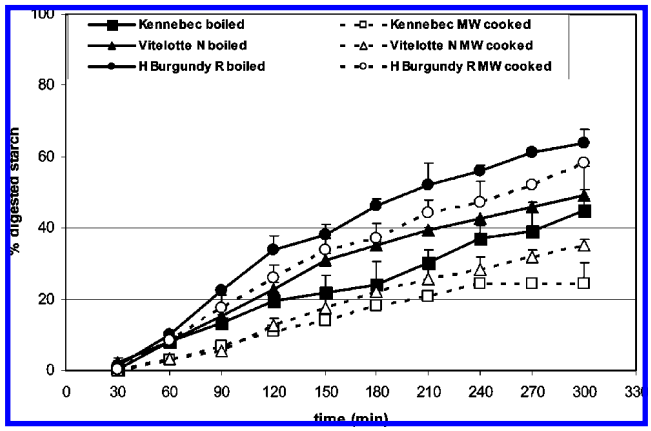


Figure 7. Starch hydrolysis curves of boiled and microwave-cooked samples of the three potato varieties.

susceptibility and the size of starch granules from different botanical origins has been highlighted; in fact, the smaller size of the starch granules resulted in a higher percentage of hydrolysis (*31*). On the contrary, in the Highland Burgundy Red variety, we observed a higher starch availability to enzymatic digestion even if in this sample we found more large-size granules. To date, the mechanism at the basis of the higher digestibility of these cooked colored cultivars remains uncertain, and a more complete chemical and physical characterization of starch granules in these colored varieties is needed to better explain these findings.

Resistant starch refers to a portion of starch and starch products that, for different reasons, become resistant to digestion in the human gastrointestinal tract. RS appears to confer health benefits to the human colonic ecosystem by its potential as prebiotic product, likely mediated by the action of butyrate, the main volatile fatty acid produced from its fermentation in the large intestine (*32*). There is also increasing interest surrounding the use of RS to enhance the fiber content of food, thus lowering its available carbohydrate content and energy value. Probably, changes observed in the microstructure of potatoes are involved not only in the rate but also in the extent of starch digestion, evaluated in this study by assessment of RS fraction (**Table 2**).

In accordance with results obtained by Niba et al. (*33*), in all of the MW-treated samples we noted RS levels higher than those of their boiled counterparts. Probably less hydrated and consequently less swollen starch granules resulted from an advanced evaporation of cellular water that, in turn, resulted in significant weight loss. Such cellular water evaporation tends to limit the gelatinization process of starch and causes the collapsing of parenchyma cells (*27*). The more packed microstructure and lower degree of gelatinization tend to limit starch availability to digestive enzymes, thus increasing RS levels.

In relation to our colored varieties of potatoes, interesting results were observed in cv. Vitelotte Noire, in which we noted higher RS contents as compared to the other two analyzed cultivars.

The analytical method used in this study to assess total and resistant starch fraction involved either α-amylase (EC 3.2.1.1) or amyloglucosidase (EC 3.2.1.3) enzymatic activities. The latter enzyme however was employed, after the elimination of products derived from α-amylase digestion by three consecutive rinsings, to complete digestion of starch residuals to glucose. It is likely that the anthocyanic fraction was also removed during rinsing and thus could not interfere in amyloglucosidase (EC 3.2.1.3) enzymatic activities. Consequently, it is likely that the high amounts of RS evaluated in cv. Vitelotte should be related mainly to the "starch structure" in this variety and, at least in part, to the amylose−amylopectin ratio in starch itself, a hypothesis that calls for further dedicated investigations.

Finally, with the aim of substantiating the inclusion of these colored varieties of potato in a healthier diet, further studies in humans are needed to reveal the potential synergistic effects between anthocyanin fraction and starch structure in modulating the glycemic index of these tubers and in sustaining the welfare of the colonic ecosystem.

ACKNOWLEDGMENT

We are grateful to Ente Cassa di Risparmio di Firenze for supplying part of the instrumentation used for this research and to Prof. Vincenzo Vecchio for the H. Burgundy Red and Kennebec samples.

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Received for review May 15, 2008. Revised manuscript received September 2, 2008. Accepted October 6, 2008. This work was partially supported by the Italian MUR (Ministero Università e Ricerca).

JF801521E