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Induction of Antioxidant Flavonol Biosynthesis in Fresh-Cut Potatoes. Effect of Domestic Cooking

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The effect of fresh-cutting and subsequent cold storage on phenolic compounds from five long-termstored potato cultivars (Agria, Cara, Liseta, Monalisa, and Spunta) was studied. Fresh-cutting induced the biosynthesis of three flavonols, which were identified by HPLC-DAD-ESIMS as quercetin 3-rutinoside, quercetin 3-diglucoside, and quercetin 3-glucosylrutinoside. The flavonols were detected after a lag period of 3 days of cold storage. The content ranged from 6 to 14 mg/100 g of fresh weight depending on the cultivar after 6 days of storage. Chlorogenic acid as the main caffeic acid derivative and the amino acids tyrosine and tryptophan were also quantified. The effect of cold storage under light or in dark was studied with new-season-harvested Monalisa potatoes. The flavonol induction was higher in fresh-cut potatoes stored under light than in the dark. However, caffeic acid derivatives were not affected. Domestic cooking such as boiling, microwaving, and frying provoked a partial loss of the flavonols, which were retained in the range of 4–16 mg per serving (213 g). Steam-cooking resulted in the highest retention of caffeic acid derivatives and aromatic amino acids compared with the other cooking methods studied. This means that due to the large amount of potatoes consumed in the Western diet, fresh-cut potatoes can be a significant source of health-promoting phenolics.

KEYWORDS: Solanum tuberosum L.; minimal processing; polyphenols; HPLC-ESIMS-MS

INTRODUCTION

Epidemiological studies have pointed out that regular consumption of fruits and vegetables imparts health benefits (1-5). Governmental institutions have recommended minimum daily intakes of fruits and vegetables because of their healthpromoting properties (6, 7). The health benefits of fruit and vegetable consumption seem to be related, at least partly, to the content of antioxidant phenolic metabolites. Therefore, there has been a renewed interest in the evaluation of the phenolic content of fruits and vegetables. The effect of technological treatments and processing in the content of these substances is a topic of major research interest (8).

Potato is one of the main vegetables consumed in European and American diets. It is always consumed after cooking (boiling, frying, baking, etc). Previous studies indicate that potato tubers contain caffeic acid derivatives (chlorogenic acid) as the main phenolic constituents (9) and that the biosynthesis of these compounds is affected by external factors such as bruising and wounding (10). An increase in chlorogenic acid content has been reported in some cultivars after bruising (11) and slicing (12). An increase in the content of chlorogenic acid and other phenolic compounds was observed in the lightexposed sliced potatoes stored for 9 days (12). The formation of polyphenols in the tubers was accompanied by the appearance of flavonoids. These researchers associated the appearance of flavonoids with the light irradiation.

Both flavonoids and caffeic acid derivatives have shown freeradical scavenging activity in vitro (13). In fact, the flavonol quercetin and its glycosides showed one of the highest antioxidant activities (14), which was associated with in vivo activity (15).

The fresh-cut industry that started out mainly as bagged lettuce has expanded to a wide variety of packaged fresh-cut fruits and vegetables. These fresh-cut products can be used as an ingredient or an entree to meet the flavor and nutritional qualities that today's consumer expects. Restaurants, fast-food establishments, and also consumers demand fresh-cut products as a good source of vitamins and antioxidants. Instead of frozen products, most consumers want fresh products that can be diced, sliced, and shredded, with top-quality taste, appearance, and high nutritional value maintained. As the popularity of fresh-cut fruits and vegetables increases, the processors need to provide tighter control over the changes that occur during the processing operations.

The aim of the present study is the evaluation of the effect of fresh-cutting and further cold storage (in the absence and presence of light) on the soluble phenolic compounds from five potato cultivars (Agria, Cara, Liseta, Monalisa, and Spunta). The fate of phenolics from fresh-cut potatoes after various domestic cooking methods will be also approached.

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MATERIALS AND METHODS

Reagents. Quercetin 3-O-rutinoside and chlorogenic acid (5-caffeoylquinic acid) were purchased from Merck (Darmstadt, Germany). Tyrosine, tryptophan, formic acid, and sodium fluoride (NaF) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO). All other reagents were of analytical grade and supplied by Merck. Milli-Q system ultrapure water (Millipore Corp., Bedford, MA) was used.

Plant Material. Long-term-stored tubers of Agria, Cara, Liseta, Monalisa, and Spunta cultivars were utilized for the first trial (i.e., induction of phenolic compounds). New-season-harvested Monalisa tubers were used for the second and third trials (i.e., light-dark storage and cooking). Potatoes were purchased from a local supplier in Murcia (Spain) and transported by car to the laboratory (5 km), where those with defects (cuttings and bruises) were discarded.

Processing and Storage Conditions. Potatoes were hand-peeled and cut in 8×8 mm strips with a manual potato cutter (Sammic CF-4, Azpeitia, Spain) at room temperature. Then, potatoes were washed with running water. Uniform strips were selected and broken pieces discarded. No postharvest chemical washing treatment was applied. Three replicates were used for each treatment and sampling date. One hundred grams of strips was selected at random from the whole bunch of potato strips and placed in 250 mL jars as one replicate. A continuous flow of humidified air at a rate of 10 mL/min was obtained by using flow boards and capillary tubing. Storage temperature was set at 4 °C. Samples were analyzed immediately after cutting (day 0) and after 1-6 and 1-7 day intervals for the first and second trials, respectively. For the first trial, a maximum light exposure of 8 h/day from two fluorescent lamps was applied. For the second trial, half of the jars were covered with aluminum foil to exclude light, whereas the other half were placed under continuous light exposition from two Osram L 58 W fluorescent lamps (München, Germany) at a 2 m distance. The photon flux density was 1 μ mol/m²/s at the level of the jars measured with a LI-COR LI-1600 steady state porometer equipped with a Quantum Q-8825 sensor (Lincoln, NE). For the cooking experiment, samples were stored under continuous light for 9 days at 4 °C.

Cooking Experiment. Four cooking methods (boiling, steamcooking, microwaving, and frying) were applied. Three replicates of 100 g of fresh strips were used for each type. Boiling and steam-cooking were applied using a pressure cooker containing 500 mL of tap water. Strips were fully dipped in water during 1.5 min for boiling, whereas strips were placed on top of the water for 2 min for steam-cooking. A microwave oven (Samsung, Cleveland, U.K.) at full power (1000 W) for 4 min was used for microwaving. Frying was done by dipping fresh strips in sunflower oil at 190 °C for 4 min. Weight loss after cooking was considered for quantification.

Extraction of Phenolic Compounds. The frozen material (5 g) was homogenized in an Ultraturrax (T-25 device, Ika-Werke, Stanfen, Germany) with 5 mL of an extraction solution of methanol/4 mM NaF in water/HCOOH (70:28.5:1.5 v/v) for 30 s on ice. The homogenate was filtered through filter cloth, centrifuged at 11340g for 10 min, and then filtered through a 0.45 μ m membrane filter (Millex-HV₁₃, Millipore Corp.) for direct analysis by HPLC after a period not exceeding 2 h.

HPLC-DAD System. Fifty microliters extracts were analyzed using an HPLC system (Merck-Hitachi, Darmstadt, Germany) equipped with a pump (model L-7100), a UV–vis detector (model L-7455), and an autosampler (model L-7200). A reversed phase C₁₈ LiChroCART column (25 × 0.4 cm; LiChrospher 100, particle size = 5 μ m) was utilized. Elution was performed using water/formic acid (19:1, v/v) (A) and HPLC-grade methanol (B) as the mobile phases, on a gradient starting with 3% B in A to reach 35% B in A at 25 min and 90% B in A at 35 min. The flow rate was 1 mL/min, and chromatograms recorded at 270, 320, and 350 nm.

HPLC-MS-MS Analyses. The HPLC system equipped with a DAD detector and mass detector in series consisted of an HPLC binary pump (G1312A), an autosampler (G1313A), a degasser (G1322A), and a photodiode array detector (G1315B) controlled by software (v. A08.03) from Agilent Technologies (Waldbronn, Germany). The mass detector was an ion-trap mass spectrometer (G2445A, Agilent Technologies) equipped with an electrospray ionization (ESI) system and controlled by a software (v. 4.0.25). Electrospray mass spectrometric analyses

were performed in the negative mode. The heated capillary and voltage were maintained at 300 °C and 4 kV, respectively. The full-scan mass spectra of the phenolic compounds were measured from m/z 100 to 1500. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, and the collision energy was set at 50%. The same column and chromatographic conditions as for the HPLC-DAD analyses were used.

Identification and Quantification of Phenolic Compounds. Phenolic compounds in potato extracts were identified by their UV spectra, recorded with a diode array detector and HPLC-ESIMS, and, wherever possible, by chromatographic comparisons with authentic markers. Flavonols were quantified as quercetin 3-*O*-rutinoside at 350 nm. Caffeic acid derivatives were quantified as chlorogenic acid at 320 nm. Tyrosine and tryptophan were quantified at 270 nm. Concentrations were expressed as milligrams per 100 g of fresh weight (fw). Repeatability of the analyses was $\pm 5\%$.

Isolation and Identification of Flavonols. The flavonols present in cv. Monalisa tubers were isolated by semipreparative HPLC equipment (Merck-Hitachi) coupled to a UV detector set at 350 nm and a reversed phase column (12.5×0.7 cm, 5 μ m particle size), using as solvents isocratic mixtures of methanol/water, with a flow rate of 1.5 mL/min. The purity of the isolated compounds was tested by analytical HPLC under the chromatographic conditions reported above.

The isolated flavonols were analyzed by UV spectrophotometry in methanol and after the addition of alkaline and metal reagents (NaOMe, AlCl₃, AlCl₃ + HCl, NaOAc, and NaOAc + H₃BO₃) (*16*). Isolated compounds were submitted to acid hydrolysis for sugar identification by thin layer chromatography (silica gel) comparisons with authentic markers (*17*).

RESULTS AND DISCUSSION

Phenolic Compounds in Cv. Monalisa Potato. HPLC chromatograms of fresh-cut Monalisa potato were characterized by the presence of two main compounds at 270 nm with DAD-UV spectra suggesting a simple phenolic structure (compound 1) and an indol structure (compound 3) (Figure 1; Table 1). Compound 2 was the main peak in the chromatogram at 320 nm and showed a typical caffeic acid derivative UV spectrum (Table 1). A remarkable increase in the caffeic acid derivative (compound 2) was observed after 6 days of storage of freshcut potato strips at 4 °C. Compound 2 became the main peak in both chromatograms (at 270 and 320 nm) (Figure 2). Compounds 1 and 3 remained at the same level as in the chromatograms obtained immediately after potato cutting. In addition, three new peaks appeared (4-6) that showed flavonollike UV spectra (UV spectra with maxima at 256 and 352 nm) (Table 1; Figure 2).

Identification of Phenolic Compounds. UV and MS-MS spectra of compounds were recorded during HPLC analysis coupled to the appropriate detectors, and the data are shown in Table 1. Compound 1 had a molecular ion of m/z 180 [M -H] coincident with that of the amino acid tyrosine. This was confirmed by cochromatography with an authentic marker. Compound 2 had a molecular ion of m/z 353 corresponding to a caffeoylquinic derivative, which coincided chromatographically with chlorogenic acid. A fragment at m/z 191 corresponding to quinic acid was detected after HPLC-MS-MS analysis of the ion at m/z 353 (Table 1). Other compounds were also detected with the same type of UV spectrum. As they were present in very small amounts, their full identification was prevented. The retention time of the first one coincided with that of cryptochlorogenic acid (4-caffeoylquinic acid). Compound 3 showed a mass of m/z 203 (M – H) that coincided with that of the amino acid tryptophan. This was confirmed by chromatographic comparison with an authentic marker. In addition, compound 3 showed the characteristic UV spectrum

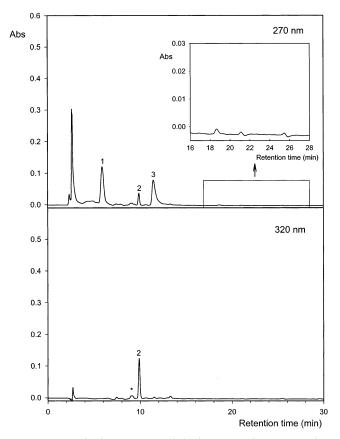


Figure 1. HPLC chromatogram of fresh-cut Monalisa potato after cutting: (1) tyrosine; (*) cryptochlorogenic acid; (2) chlorogenic acid; (3) tryptophan. (Insert) Enlargement of the time range in which flavonols are detected.

of indol-nucleus, similar to those observed for indol-glucosinolates (18).

Compounds 4-6 showed UV spectra similar to those of quercetin derivatives (16). Their HPLC-MS analysis showed that 4 was a quercetin deoxyhexosyldihexoside (M - H, m/z771). Compound 5 was a quercetin dihexoside (M – H, m/z625), and compound 6 was quercetin deoxyhexosylhexoside (M - H, m/z 609). The last one coincided chromatographically with quercetin 3-rutinoside [quercetin 3-rhamnosyl(1-6)glucoside]. HPLC MS-MS analysis after ion isolation showed that compound 4 first lost a hexosyl residue (M - 162), indicating that in this compound a hexoxyl is the terminal sugar. The occurrence of a fragment at m/z 609, coincident with quercetin 3-rutinoside, confirmed that this was a quercetin 3 (hexosylrutinoside). Compound 5 had two hexosyl residues on a quercetin molecule (M - H, m/z 625). HPLC MS-MS analysis of the isolated ion 625 showed an intermediate ion at m/z 463 corresponding to quercetin hexoside and the corresponding quercetin (3,5,7,3',4'-pentahydroxyflavone) aglycon ion at m/z301. Compound 6, which coincided chromatographically with

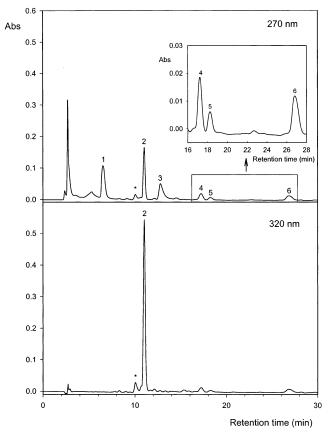


Figure 2. HPLC chromatogram of fresh-cut Monalisa potato after 6 days at 4 °C: (1) tyrosine; (*) cryptochlorogenic acid; (2) chlorogenic acid; (3) tryptophan; (4) quercetin 3-glucosylrutinoside; (5) quercetin 3-diglucoside; (6) quercetin 3-rutinoside. (Insert) Enlargement of the time range in which flavonols are detected.

rutin (quercetin rutinoside), under HPLC MS-MS analysis showed a clear loss of a deoxyhexosyl (rhamnosyl) residue. The intermediate ion at m/z 463 (quercetin hexoside) and the corresponding aglycon at m/z 301 were also detected. These flavonols were isolated by semipreparative HPLC. Their UV study in methanol and after the addition of alkaline and metal reagents [λ_{max} MeOH, 352, 266sh, 256 nm; +NaOMe, 401 (increased absorbance), 325, 270 nm; +AlCl₃, 418, 272 nm; +AlCl₃ + HCl, 344, 288, 269 nm; +NaOAc, 372, 321, 272 nm; +NaOAc + H₃BO₃, 372, 260 nm] showed that all of them presented an identical substitution pattern of the phenolic hydroxyls in the quercetin molecule. In all cases, this study showed that quercetin was substituted at the 3-position, with free hydroxyls at the 5-, 7-, 3'-, and 4'-positions of the flavonoid nucleus (16). The aglycon quercetin was detected in all cases after acid hydrolysis of these compounds. The sugars glucose and rhamnose were detected in compound 4, glucose was detected in compound 5, and glucose and rhamnose were detected in compound 6. This indicated that 4 was quercetin

Table 1. HPLC-DAD and HPLC-MS-MS of Fresh-Cut Cv. Monalisa Potato Phenolics

peak	HPLC-UV-DAD (nm)	HPLC-ESIMS (m/z)	MS-MS ^a (<i>m</i> / <i>z</i>)	structure		
1	274, 280sh ^b	180 (M – H)		tyrosine		
2	298sh, 325	353 (M – H)	191	chlorogenic acid		
3	258sh, 270, 278, 288, 322	203		tryptophan		
4	256, 266sh, 294sh, 352	771 (M – H)	609, 591, 301	quercetin 3-glucosylrutinoside		
5	256, 266sh, 296sh, 352	625 (M – H)	463, 301	quercetin 3-diglucoside		
6	256, 266sh, 294sh, 352	609 (M – H)	463, 301	quercetin 3-rutinoside		

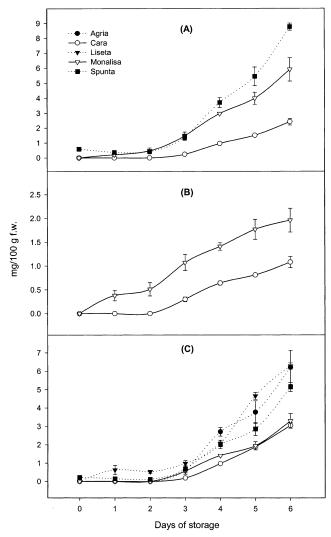


Figure 3. Flavonol induction in fresh-cut potato cultivars during storage for 6 days at 4 °C: (A) quercetin 3-glucosylrutinoside; (B) quercetin 3-diglucoside; (C) quercetin 3-rutinoside.

3-glucosylrutinoside, **5** was quercetin 3-diglucoside, and **6** was quercetin 3-rutinoside (rhamnsoylglucoside).

Flavonol and Chlorogenic Acid Induction in Potato Cultivars. The induction of these three flavonols was studied in four potato cultivars (Agria, Cara, Liseta, and Spunta) and was compared to that of cv. Monalisa. The induction of flavonols as well as the evolution during storage varied depending on the cultivar. Quercetin 3-glucosylrutinoside reached contents of approximately 9, 6, and 2 mg/100 g of fw in Spunta, Monalisa, and Cara, respectively (Figure 3A). Quercetin 3-diglucoside was only induced in small amounts in cultivars Monalisa and Cara (2 and 1 mg/100 g of fw, respectively) (Figure 3B). Quercetin 3-rutinoside was induced in all cultivars after cutting and reached values as high as 6 mg/100 g of fw in Agria and Liseta and as small as 3 mg/100 g of fw in Cara and Monalisa after 6 days of storage (Figure 3C). Flavonol biosynthesis was apparent in potato strips after a lag period of 3 days of storage and then increased steadily during the following 3 days (Figure 4A). Considering the total flavonols induced by the fresh-cut process in potatoes, Spunta was the cultivar that accumulated the highest flavonol content (~14 mg/100 g of fw). Monalisa accumulated 11 mg/100 g of fw, and the cultivars Agria, Cara, and Liseta induced similar amounts of flavonols (6 mg/100 g of fw) (Figure 4A).

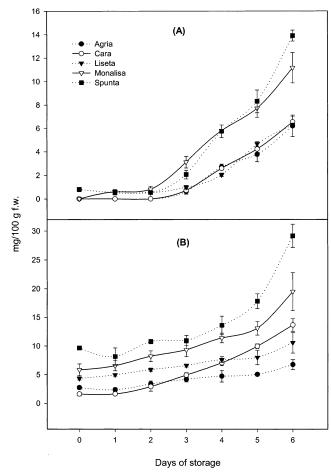


Figure 4. Kinetics of total flavonol (A) and caffeic acid derivative (B) induction in fresh-cut potato cultivars at 4 °C.

A large difference in caffeic acid derivative (chlorogenic and related compounds) content among the studied potato cultivars at harvest was found (Figure 4B). Cara showed the lowest content (2 mg/100 g of fw), followed by Agria < Liseta < Monalisa. The cultivar Spunta contained the highest caffeic acid content (10 mg/100 g of fw). Regarding the induction kinetics of caffeic acid derivatives, the biosynthesis started slowly during the first 3 days of storage. Then, it increased rapidly after the fourth day in Spunta, Monalisa, and Cara to reach 30, 20, and 14 mg/100 g of fw, respectively, at the end of the storage. On the other hand, Agria and Liseta strips showed a moderate increase of caffeic acid derivatives throughout the storage to reach contents of 7 and 10 mg/100 g of fw, respectively. The increase in caffeic acid derivatives was expected, as this type of phenolic metabolite is induced by wounding in different plant sources (19, 20) including potato (21) through the induction of the enzyme phenylalanine ammonia-lyase (PAL). This induction is related to the wound-healing process in order to fight pathogen attack after tissue wounding (22). It should be stressed that the induction of phenolic compounds can increase the velocity of oxidative reactions catalyzed by enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) giving rise to melanins (enzymatic browning). The induction of phenolics is a key factor in tissues that are devoid of phenolics such as the white tissue of lettuce, the so-called midrib. In this case, phenolics accumulation (caffeic acid derivatives) is the rate-limiting step in the enzymatic browning (23). However, in potato (as in other sources) the initial content of phenolic compounds (mainly chlorogenic acid) is high enough to provoke browning so that

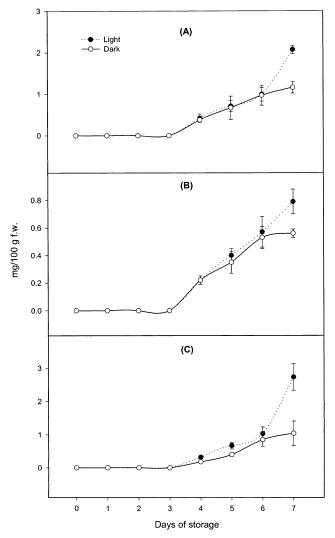


Figure 5. Effect of storage under light or dark conditions on the woundinduced flavonols in fresh-cut Monalisa potato: (A) quercetin 3-glucosylrutinoside; (B) quercetin 3-diglucoside; (C) quercetin 3-rutinoside.

phenolics accumulation is not the rate-limiting step in browning development (21).

The aromatic amino acids tyrosine and tryptophan were not induced by cutting. They showed similar contents (or even a slight decrease in some cultivars) during the whole storage period of fresh-cut potatoes (data not shown). The tyrosine content ranged from \sim 50 mg/100 g of fw in Agria and Monalisa to 15 mg/100 g in Spunta and Cara. In the case of tryptophan, smaller contents were found with values between 1 and 4 mg/100 g of fresh-cut potatoes.

The amino acid tyrosine, which is a phenolic compound (4hydroxyphenylalanine), and the inducible potato phenolics (flavonols and caffeic acid derivatives) share a number of biosynthetic steps catalyzed by inducible enzymes such as DHAP synthase (EC 4.1.2.15), chorismate mutase (EC 5.4.99.5), and others (19). However, there are other specific enzymes in the tyrosine biosynthesis such as phenylalanine 4-hydroxylase (EC 1.14.16.1), which catalyzes the para-hydroxylation of phenylalanine to yield tyrosine, as well as enzymes that are involved in tyrosine biosynthesis from prephenate via 4-hydroxylphenylpyruvate (19). Perhaps these enzymes are not inducible by wounding, which could explain the noninduction of tyrosine after fresh-cutting.

Previous studies have linked flavonoid biosynthesis to light irradiation (12). Therefore, the effect of light on the observed flavonoid induction was also approached.

Effect of Light on Wound-Induced Phenolic Biosynthesis. Cv. Monalisa potato was selected as it induced the three flavonols in significant amount. Flavonol biosynthesis started after 3 days of storage under both dark and light conditions, with similar rates and slopes (Figure 5). The most significant difference between dark and light storage conditions occurred after 6 days, at which time light-exposed strips showed a higher rate of flavonol accumulation. This was clearly noted when total flavonols were considered (Figure 6A). After 7 storage days, potato strips accumulated 3 mg/100 g of fw of flavonols in the dark, whereas flavonols reached 6 mg/100 g when stored under light. In both storage conditions, caffeic acid derivatives reached values of $\sim 14 \text{ mg}/100 \text{ g}$ after 7 days (Figure 6B). In addition, light did not show any effect on the tyrosine and tryptophan contents either (data not shown). These results confirmed the induction of phenolic compounds in potato strips by freshcutting. Laanest et al. (12) observed the appearance of flavonoids in sliced potato and associated this increase to the light irradiation. The accumulation of phenolic compounds in freshcut products could be related to the defense mechanism as it has been described in shredded carrots (24, 25). To the best of our knowledge, the biosynthesis of flavonols by fresh-cutting has never been described before.

On the other hand, the content of induced phenolic compounds in the case of new-season potato tubers was much lower than when long-term potato tubers were assayed. New-seasonharvested Monalisa tubers contained lower amounts of individual flavonols as well as caffeic acid derivatives than long-termstored potatoes (**Figures 3–6**).

Effect of Cooking on Phenolic Composition. Significant differences were observed in the content of flavonols, caffeic acid derivatives, and aromatic amino acids in the cooked potato strips when compared to uncooked ones (Table 2). Each individual flavonol decreased after cooking to half of their initial content in all treatments. No significant differences among cooking processes were observed. The individual and total flavonols were still present in important amounts in the cooked potato strips. The flavonoid loss was significantly pronounced

Table 2. Effect of Cooking on Individual and Total Flavonols, Caffeic Acid Derivatives, Tyrosine, and Tryptophan in Fresh-Cut Cv. Monalisa Potato^a

cooking treatment ^b	quercetin 3-glucosylrutinoside	quercetin 3-diglucoside	quercetin 3-rutinoside	total flavonoids	caffeic acid derivatives	tyrosine	tryptophan
uncooked potato	2.6	0.9	3.6	7.1	17.4	11.5	2.5
boiling	1.2	0.4	2.4	4.0	5.9	2.8	0.7
steam-cooking	1.5	0.5	2.0	4.0	8.4	5.9	1.3
microwaving	1.1	0.4	1.5	3.1	6.7	2.9	0.6
frying	1.1	0.4	1.8	3.3	4.9	3.8	0.7
LSD (5%)	0.3	0.1	0.5	0.9	1.3	0.6	0.2

^a Values are the mean (n = 3) in mg/100 g of fw. Mean values were compared using the least significant differences test (LSD). ^b See Materials and Methods for cooking identification.

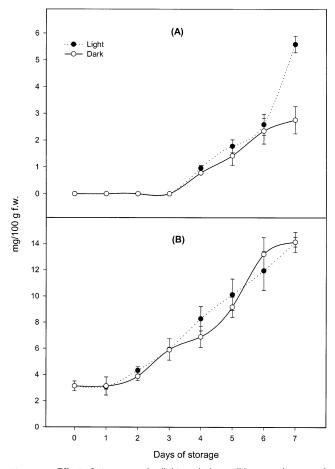


Figure 6. Effect of storage under light or dark conditions on the woundinduced flavonols (A) and caffeic acid derivatives (B) in fresh-cut Monalisa potato.

during microwaving and frying (Table 2). In the case of caffeic acid derivatives, half of their initial content was retained after steam-cooking, whereas only one-third of the original value was detected in the cases of boiling and frying (Table 2). The effects of the different cooking processes on chlorogenic acid were also studied, because it constituted up to 70% of the total phenolic content of fresh-cut potato. Steam-cooked potato strips retained 42% of the initial chlorogenic acid, whereas only 24% was preserved after frying. Friedman (10) previously reported that oven-baked, French-fried, and mashed potato contained no chlorogenic acid, whereas boiled and microwaved potato retained 35 and 55%, respectively, of the original amount. The differences observed in the loss of chlorogenic acid with our results could be due to both temperature and time of cooking processes as well as to the type of cutting (strips, slices, etc). In the case of aromatic amino acids, steam-cooking preserved >50% of the initial content, whereas boiling, microwaving, and frying produced higher losses (~75%) (Table 2). Steam-cooking was selected as the best cooking method because it retained the largest content of flavonoids and other polyphenols.

These compounds, although in small amounts, can be significant from a dietary point of view. Large servings are consumed (a medium size serving is 213 g according to the USDA) (26), and the annual consumptions per capita are around 42 and 45 kg in the United States and Spain, respectively (27, 28). As most cooking treatments preserve at least 50% of both induced flavonols and caffeic acid derivatives, amounts of 4-16 mg of quercetin derivatives and 6-30 mg of caffeic acid derivatives are especially

significant when compared with other flavonoid-rich vegetables, as in the case of onion. Onions are one of the main natural sources of flavonols in the diet (185–330 mg of quercetin/kg of fw) (29), although their dietary significance is relatively low when compared to that of potato as the per capita consumption is as small as 0.3 kg per year in the United States (26).

In addition, although cooking decreases the total flavonoid and caffeic acid derivative contents of the potato, this does not mean that cooking cannot exert an overall positive effect on flavonoid bioavailability, as has been shown for other phytochemicals in which cooking has a positive effect on compounds released from the food matrix in the gastrointestinal tract and their further absorption in the intestine, as is the case of lycopene in tomato (*30*) and ellagic acid in strawberry (*31*).

As a conclusion, fresh-cutting and subsequent storage of potatoes can induce the accumulation of flavonols, and a significant part of them are preserved in the food after cooking. Due to the relatively large amounts of potatoes consumed in Western diets, fresh-cut potatoes can be a significant source of health-promoting polyphenols. In this way, technologies that preserve the antioxidant content of fresh-cut products or even enhance the nutritional content would be a major research goal.

LITERATURE CITED

- (1) Arai, Y.; Watanabe, S.; Kimira, M.; Shimoi, K.; Mochizuki, R.; Kinae, N. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol. *J. Nutr.* **2000**, *130*, 2378– 2383.
- (2) Cao, G.; Booth, S. L.; Sadowski, J. A.; Prior, R. L. Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. *Am. J. Clin. Nutr.* **1998**, *68*, 1081–1087.
- (3) Lampe, J. W. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. Am. J. Clin. Nutr. 1999, 70, 475–490.
- (4) Liu, S.; Manson, J. E.; Lee, I. M.; Cole, S. R.; Hennekens, C. H.; Willet, W. C.; Buring, J. E. Fruit and vegetable intake and risk of cardiovascular disease: the women's health study. *Am. J. Clin. Nutr.* 2000, 72, 922–928.
- (5) Tavani, A.; La-Vecchia, C. Fruit and vegetable consumption and cancer risk in a Mediterranean population. *Am. J. Clin. Nutr.* **1995**, *61*, 1374–1377.
- (6) Fruit and vegetables for health: http://www.fruitveg.com/sp/ welcome.html.
- (7) Eat5aday for better health: http://www.5aday.com/.
- (8) Tomás-Barberán, F. A.; Espín, J. C. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J. Sci. Food Agric. 2001, 81, 853–876.
- (9) Brandl, W.; Herrmann, K. On the occurrence of chlorogenic acids in potatoes. Z. Lebensm. Unters.-Forsch. 1984, 178, 192–194.
- (10) Friedman, M. Chemistry, biochemistry, and dietary role of potato polyphenols. A review. J. Agric. Food Chem. 1997, 45, 1523– 1540.
- (11) Dale, M. F. B.; Griffiths, D. W.; Bain, H. Effect of bruising on the total glycoalkaloid and chlorogenic acid content of potato (*Solanum tuberosum*) tubers of five cultivars. *J. Sci. Food Agric.* **1998**, 77, 499–505.
- (12) Laanest, L.; Tohver, A.; Palm, E. Soluble phenolics and alicyclic acids in aging potato tuber slices. *Eesti Tead. Akad. Toim., Biol.* **1995**, *44*, 1–10.
- (13) Chen, J. H.; Ho, C. T. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. J. Agric. Food Chem. 1997, 45, 2374–2378.
- (14) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: Structure–activity relationships. *Free Radical Biol. Med.* **1997**, *22*, 749–760.

- (16) Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: Berlin, Germany, 1970.
- (17) Kartnig, T.; Wegschaider, O. Eine m-glichkeit identifizierung von zuckern aus kleinsten mengen von glykosides oder aus zuckergemischen. J. Chromatogr. 1971, 61, 375–377.
- (18) Kiddle, G.; Bennett, R. N.; Botting, N. P.; Davidson, N. E.; Robertson, A. A. B.; Wallsgrove, R. M. High-performance liquid chromatographic separation of natural and synthetic desulphoglucosinolates and their chemical validation by UV, NMR and chemical ionization-MS methods. *Phytochem. Anal.* 2001, *12*, 226–242.
- (19) Diallinas, G.; Kanellis, A. K. A phenylalanine ammonia-lyase gene from melon fruit: cDNA cloning, sequence and expression in response to development and wounding. *Plant Mol. Biol.* **1994**, 26, 473–479.
- (20) Saltveit, M. E. Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharvest Biol. Technol.* 2000, 21, 61–69.
- (21) Cantos, E.; Tudela, J. A.; Gil, M. I.; Espín, J. C. Phenolic compounds and related enzymes are not rate-limiting in browning development of fresh-cut potatoes. *J. Agric. Food Chem.* 2002, 50, 3015–3023.
- (22) Dixon, R. A.; Paiva, N. L. Stress-induced phenylpropanoid metabolism. *Plant Cell* **1995**, 7, 1085–1097.
- (23) Tomás-Barberán, F. A.; Gil, M. I.; Castañer, M.; Artés, F.; Saltveit, M. E. Effect of selected browning inhibitors on phenolic metabolism in stem tissue of harvested lettuce. *J. Agric. Food Chem.* **1997**, *45*, 583–589.
- (24) Babic, I.; Amiot, M. J.; Nguyen-the, C.; Aubert, S. Changes in phenolic content in fresh ready-to-use shredded carrots during storage. J. Agric. Food Chem. 1993, 41, 351–356.

- (25) Babic, I.; Amiot, M. J.; Nguyen-the, C.; Aubert, S. Accumulation of chlorogenic acid in shredded carrots during storage in oriented polypropylene film. J. Agric. Food Chem. **1993**, 41, 840–841.
- (26) USDA, Agricultural Research Service. USDA Nutrient Database for Standard Reference, release 13; 1999; nutrient data laboratory home page, http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl.
- (27) Data per capita food consumption data system: http://www. ers.usda.gov/data/foodconsumption/spreadsheets.asp.
- (28) MAPYA. La Alimentación en España; Ministerio de Agricultura Pesca y Alimentación: Spain, 1995.
- (29) Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black, C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery. *J. Agric. Food Chem.* **1997**, *45*, 590–595.
- (30) van den Berg, H.; Faulks, R.; Granado, F.; Hirschberg, J.; Olmedilla, B.; Sandmann, G.; Southon, S.; Stahl, W. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *J. Sci. Food Agric.* **2000**, *48*, 880– 912.
- (31) Zafrilla, P.; Ferreres, F.; Tomás-Barberán, F. A. Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry (*Rubus idaeus*) jams. J. Sci. Food Agric. 2001, 49, 3651–3655.

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