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L-Galactono-γ-Lactone Dehydrogenase Activity and Vitamin C Content in Fresh-Cut Potatoes Stored under Controlled Atmospheres

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L-Galactono-γ-lactone dehydrogenase (GLDH) activity and vitamin C content as ascorbic acid (AA) plus dehydroascorbic acid (DHA) were evaluated in five potato tuber cultivars (Agata, Altesse, Franceline, Manon, and Monalisa). The effect of fresh-cutting and subsequent refrigerated storage of Manon potato under different atmospheres (air, 20% CO_2 + air, 100% N_2, and vacuum packaging) on GLDH activity and vitamin C content was also determined. GLDH from the five potato tuber cultivars showed typical inhibition kinetics by high substrate concentration in the synthesis of AA from its physiological precursor L-galactonic acid-γ-lactone (GL). GLDH activity was not correlated with the corresponding vitamin C content in any potato tuber cultivar. GLDH from all the cultivars presented a major isoform with isoelectric point (IEP) 5, which changed to IEP = 4.3 after minimal processing. In addition, the GLDH-catalyzed synthesis of AA by the new isoform showed typical Michaelis kinetics, in which the enzyme became more efficient to catalyze the reaction. Whether the change in the isoform pattern was due to either post-translational modifications or de novo synthesis of a new isoenzyme remains unanswered. Fresh-cutting increased GLDH activity from 4.7-fold (vacuum packaging) to 11-fold (air) after 6 days. In addition, 100% of vitamin C content was retained in air and decreased in the rest of atmospheres after this storage period, following the sequence vacuum packaging (89%) > 100% N_2 (78%) > 20% CO_2 + air (63%). This tendency was correlated with the corresponding GLDH activity detected in each storage atmosphere, except in the case of 20% CO_2 + air. Vacuum packaging proved to be the best storage condition, because fresh-cut potatoes did not turn brown and retained 89% of initial vitamin C content.

KEYWORDS: L-Galactono-γ-lactone dehydrogenase (GLDH); fresh-cut potato; ascorbic acid; vitamin C; enzyme kinetics; controlled atmosphere; minimal processing; vacuum packaging

INTRODUCTION

Reactive oxygen species (ROS) cause oxidative stress, which alters cellular structures and cell death processes in humans, leading to aging and a number of pathologies, including cancer as well as cardiovascular and neurodegenerative diseases (*1*). Therefore, there is great interest in the health-promoting effects of antioxidants that prevent oxidative stress by scavenging ROS (*2*).

The main human dietary antioxidants are vitamins E and C, phenolic compounds, and carotenoids. Ascorbic acid (AA) is the most active form of vitamin C, and it is quantitatively the predominant antioxidant in plant cells, where it plays an important role in growth and metabolism and acts as a free radical scavenger (*3*−*5*). Ascorbate is oxidized by ROS to yield monodehydroascorbate, which forms dehydroascorbic acid (DHA). DHA also exhibits biological activity, because it can be readily converted into AA in the human body (*6*).

Potatoes play an important role in the diet, because it is the most consumed vegetable in many countries (*7*). Despite their moderate AA content (10−30 mg/100 g fresh weight), potatoes are a major source of AA in the Western diet, because of the large amounts consumed (*8*).

There is increasing consumer demand for fresh-cut fruits and vegetables, including fresh-cut potatoes, as a source of vitamins and antioxidants. However, controls during fresh-cut processing are essential, because antioxidants such as phenolics and AA are affected when bruising, trimming, and cutting of fruits and vegetables occurs (*9*, *10*). Therefore, research should focus on

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the optimal maintenance of both nutritional and sensorial qualities of fresh-cut products. In this context, a recent study has reported the retention of vitamin C in fresh-cut potatoes stored in air, whereas vitamin C content decreased in fresh-cut potatoes stored under modified atmosphere packaging (*10*). This work demonstrated the biosynthesis and accumulation of vitamin C when fresh-cut potatoes were stored in various atmospheres, and the authors suggested that this phenomena could be related to changes in the enzyme activity of L-galactono-γ-lactone dehydrogenase (GLDH, EC 1.3.2.3), which catalyzes the final step of AA biosynthesis (*11*, *12*).

Despite the importance of AA in both plant and human physiology, the metabolic pathway leading to AA biosynthesis is not yet fully understood. In fact, the last step in the biosynthesis pathway has been recently elucidated in plants, involving the conversion of L-galactonic acid-γ-lactone (GL) to ascorbic acid in a GLDH-catalyzed reaction (*4*, *13*). GLDH is bound to the inner mitochondrial membrane (*11*, *12*, *14*) and is highly specific for the AA precursor GL. This enzyme has been previously studied in a few sources, such as white and sweet potato (*11*, *15*), spinach (*12*), cauliflower (*16*), sweet pepper (*17*), and kidney bean (*14*). However, there are no previous studies concerning the effect of minimal processing (cutting and cold storage in different atmospheres) on this enzyme and the possible relationship with AA content.

Therefore, the aim of the present work is to study the kinetic characterization of the enzyme GLDH in five potato cultivars (Agata, Altesse, Franceline, Manon, and Monalisa) and its correlation with initial AA content in potato tuber. In addition, GLDH activity, vitamin C content, and browning severity were evaluated in the cultivar Manon upon cutting and cold storage in different controlled atmospheres (i.e., air (AIR), high CO_2 concentration (20% CO_2 + AIR), 100% N_2, and vacuum packaging (VAC)).

MATERIALS AND METHODS

Reagents. AA, bovine serum albumin (BSA), citric acid, L-cysteine, cytochrome C (Cyt *c*), dehydroascorbic acid (DHA), GL, 1,2-phenylenediamine dihydrochloride (OPDA), ethylenediaminetetraacetic disodium salt (EDTA), mannitol, 3-[*N*-morpholino] propanesulfonic acid (MOPS), sodium cyanide (NaCN), sodium fluoride (NaF), nitro blue tetrazolium chloride (NBT; 3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-bis[2-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride]), Triton X-100 (TX-100) were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical grade and supplied by Merck (Darmstadt, Germany). Milli-Q system ultrapure water (Millipore Corp., USA) was used throughout this research.

Plant Material. Agata, Altesse, Franceline, Manon, and Monalisa potatoes were selected because of their availability throughout the present experiment. These potatoes were used for the screening of cultivars (initial GLDH activity and AA content). The Manon cultivar was selected to evaluate the effect of fresh-cutting and storage under controlled atmospheres on both GLDH activity and vitamin C content. Altesse tubers were harvested at the middle of August, while Agata, Franceline, Manon, and Monalisa were harvested in September-October. After harvest, all potatoes were stored at 7−8 °C and 90% relative humidity (RH). Tubers were supplied by Agroinnova S. L. (Tordesillas, Spain) and transported by car to the laboratory in less than 12 h, where those with defects (cuts and bruises) were discarded. At the laboratory, sound tubers were kept at 4 °C and 70% RH in darkness for 2 days before processing. Each potato cultivar assayed here included a complete certificate of "traceability" which specified all the aspects and factors concerning the cultivar: agronomic and climatic conditions (soil, type and doses of fertilization, irrigation, pesticides, herbicides, temperature, etc.), analysis at harvest (starch, dry matter, nitrates, etc.), culinary tests (frying, cooking, etc.), size, etc.

Processing and Storage Atmospheres. Potatoes were hand-peeled and washed with running water. After peeling, the tubers were sorted for absence of visual defects and uniform color. Potatoes were cut in 8×8 mm^2 strips with a manual potato cutter (Sammic CF-4, Azpeitia, Spain) at room temperature. The strips were washed again with running water and then strained in a draining board to remove surface water. Uniform strips were selected and broken pieces discarded. No postharvest chemical washing treatment was applied. One hundred grams of strips were selected at random from the whole bunch of fresh-cut potato strips as one replicate. Three replicates were used for each treatment and sampling date. Four different storage atmospheres were studied: storage in air (AIR), controlled atmosphere of 20% CO_2 + air (CA 20% CO_2), controlled atmosphere of 100% N_2 (CA 100% N_2) and vacuum packaging (VAC).

For AIR, CA 20% CO_2, and CA 100% N_2 atmospheres, fresh-cut potato strips were placed in 250-mL jars as one replicate. A continuous humidified flow at a rate of 15 mL/min was obtained by using flow boards with needle valves. Changes in O_2 and CO_2 concentrations in AIR, CA 20% CO_2, and CA 100% N_2 were monitored daily, using a gas chromatograph (Perkin-Elmer autosystem, Connecticut) equipped with a thermal conductivity detector.

For VAC, multilayer film bags (BB4L Cryovac, Sealed Air S. L., Sant Boi de Llobregat, Spain) were used. The film characteristics were as follows: 59-μm thickness and permeance at 23 °C and 0% RH of 1.4×10^{-13} mol · s^{-1} · m^{-2} · Pa^{-1} for O_2 and 7.1×10^{-13} mol · s^{-1} · m^{-2} · Pa^{-1} for CO_2. The film physical properties are similar to the polymers used in the fresh-cut potato industry. A gas exchange device with a vacuum packaging machine (Zermat, Carburos Metálicos S. A., Madrid, Spain) was utilized. Vacuum packaging was carried out by exclusion of air from the bags.

Sensory Evaluation. Browning of fresh-cut potatoes was evaluated immediately after cutting and after 1, 2, 3, 4, 5, and 6 day intervals by a panel of five judges. Evaluation was scored on a 3-point scale (1 = no browning; 2 = moderate; 3 = severe).

Ascorbate Extracts and HPLC Analysis. AA and DHA contents were determined by the method of Zapata and Dufour (*18*), with some modifications previously reported by Tudela et al. (*10*).

Ascorbate content was determined in all the potato tuber cultivars (hereafter termed as "initial AA"). In addition, ascorbate was also evaluated daily, during six storage days, for Manon cultivar. Ascorbate analysis was performed in triplicate. Mean values ± standard deviation are shown.

Preparation of Mitochondria-Enriched Potato Extracts. The extracts were obtained according to the method of Struglics et al. (*19*), with some modifications. Fifty grams of potato were added to 50 mL of extraction medium (0.9 M mannitol, 30 mM MOPS, 3mM EDTA, 25 mM L-cysteine, and 0.3% (w/v) BSA). The pH was adjusted to 7.3 by dropwise addition of 8 M KOH. The mixture was homogenized with five consecutive pulses for 3 s in an Osterizer blender (Sunbeam, Delray Beach, FL) and further filtered through nylon gauze (120-μm mesh). The homogenate was left standing for 5 min, allowing the starch to sediment, and then centrifuged at 1600*g* for 15 min in a Beckman J2−21 centrifuge (Altberville, MN), using a Beckman JA-14 rotor. The supernatant was decanted and centrifuged at 12800*g* for 25 min. The pellets were redissolved in 30 mL of washing medium (0.3 M mannitol, 10 mM MOPS, 1mM EDTA, and 0.1% (w/v) BSA, pH 7.2) and centrifuged at 17400*g* for 10 min, using a Beckman JA-20 rotor. Every final pellet (8 pellets) enriched in mitochondria was redissolved in 300 μL of washing medium without BSA (0.3 M mannitol, 10 mM MOPS, and 1 mM EDTA). This means that every extraction assay yielded a total volume of 2.4 mL of mitochondrial-enriched extract.

The extraction protocol was repeated three times for each cultivar. In addition, the protocol was also repeated three times for each treatment and storage day, in the case of the Manon cultivar, to evaluate the effect of cutting and storage conditions on GLDH activity.

GLDH Assay. GLDH activity was measured at 25 °C, according to the method of Imai et al. (*20*), with minor modifications. NaCN was included to prevent reoxidation of reduced Cyt *c* by the Cyt *c* oxidase present in the extract. Cyt *c* is used as a coupled reagent to determine

GLDH activity. The precursor GL is converted to ascorbic acid, which reduces Cyt c (20), with the subsequent change in Cyt c spectrum and corresponding absorbance (reduced Cyt c has a maximum at 550 nm). No effect of cyanide was observed on GLDH activity, in accordance with previous reports (15). The enzyme preparation with GLDH activity was constituted by 200 μL of the mitochondria-enriched extract and 200 μL of a solution containing 0.2% TX-100 and 50 mM sodium phosphate buffer (PB) pH 8 (solution A). After vigorous shaking, the enzyme preparation was left for 2 h at 4 °C until its assay, to release the enzyme from the mitochondrial inner membrane. The standard reaction mixture for determining GLDH activity contained 60 μM Cyt c, 25 mM PB pH 8.0, 1 mM GL, 60 μM NaCN, and 20 μL of enzyme preparation (6 μg protein) in a total volume of 1 mL. GLDH activity was quantified as the Cyt c reduction rate at 550 nm ($\epsilon_{550\ nm}$ = 29 500 $M^{-1}cm^{-1}$) (21). The linear increase of absorbance at 550 nm was recorded immediately after the addition of the substrate GL. The linear regression of the spectrophotometric recording rendered GLDH activity. No activity was detected in the absence of GL as well as without previous incubation with solution (A). One unit of enzyme activity was defined as the amount of extract required to oxidize 1 μmol GL (equivalent to the formation of 2 μmol reduced Cyt c) per min. GLDH activity was measured in freshly prepared extracts. It should be stressed that GLDH activity was lost after 3 days in both fresh (4 °C) and frozen (−20 °C) extracts (with or without TX-100 treatment), which should be taken into account in the characterization of this enzyme.

GLDH activity from potato tuber (i.e., before cutting) is hereafter termed as "initial" GLDH activity.

The spectrophotometric assays for determining GLDH activity were recorded in a UV-1603 Shimadzu spectrophotometer (Tokyo, Japan). Temperature was controlled at 25 °C with a temperature controller (CPS 240A Shimadzu), checked using a precision of ±0.1 °C.

Kinetic Data Analysis. The apparent V_m (maximum rate), K_m (Michaelis constant), and K_{SI} (substrate inhibition constant) values were calculated from triplicate measurements of the rate for each initial substrate (GL) concentration, using three different extracts of each potato cultivar. The mean value of each kinetic constant is shown.

Substrate inhibition constants (K_{SI}) were calculated by using the equation

$$v = \frac{V_m\,[S]}{K_m + [S]\left(1 + \frac{S}{K_{SI}}\right)} \tag{1}$$

(22), where [S] is the GLDH substrate (GL) concentration.

Nonlinear regression fittings were carried out by using the Marquardt−Levenberg algorithm (23), implemented in the Sigma Plot 6.0 program for Windows (SPSS Science, Chicago, USA). Coefficient of variation (C_V = (mean/SD) * 100) was always less than 10% in the determination of the above kinetic values.

Protein Assay. Protein concentrations were determined by the method of Bradford (24) using bovine serum albumin as a standard.

Isoelectric Focusing (IEF) Experiments. Enzymatic extracts were obtained as described above. A sample of 1 mL of extract was mixed with 1 mL of solution A and left for 2 h at 4 °C, to release the enzyme from the membrane. Afterward, the mixture was concentrated by using Microcon YM-10 filter membrane (Millipore Corporation, USA), and 4 μL of the concentrated sample were applied to the gel. Isoelectric points (IEP) of GLDH were determined in the electrophoresis unit Phast System (Pharmacia, Sweden). Isoelectric focusing gels (PhastGel from Pharmacia) with IEP range of 3−9 were used. Conditions for running IEF experiments were those suggested by the manufacturer. Broad IEP (3−10) kit markers from Pharmacia were used.

The gels were rinsed with 15 mM PB pH 8 and further incubated with a mixture containing 150 μM NaCN, 15 mM PB pH 8, 1 NBT mM, and 1 mM GL, to develop enzyme activity. GHLD activity was visualized by the appearance of blue bands in the gel. No bands were detected in the gels in the absence of the substrate GL. Stained gels were immediately scanned. Some faint bands were lost during image processing.

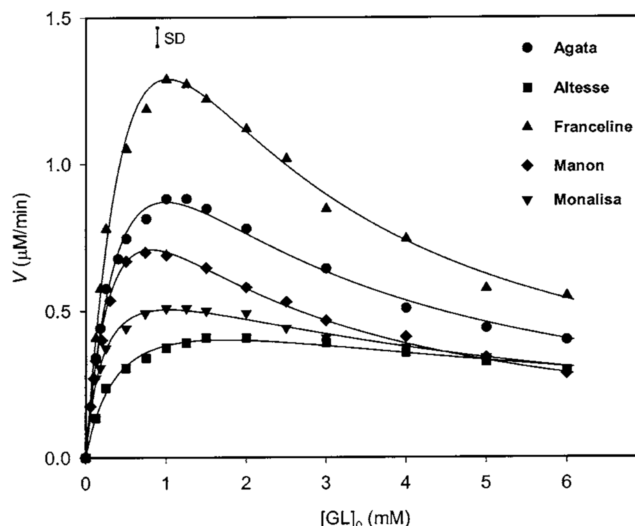

Figure 1. High substrate inhibition kinetics of GLDH from potato tuber. The reaction mixture contained 60 μM Cyt c, 25 mM PB pH 8.0, 60 μM NaCN, 20 μL of enzyme preparation (6 μg protein), and increasing GL concentrations from 0.12 to 6 mM at 25 °C. Total assay volume was 1 mL. The vertical lane indicates the average value of standard deviations (SD). Symbols designate the experimental data and solid lines the nonlinear regression fitting of experimental data to eq 1 (see Materials and Methods for details).

RESULTS AND DISCUSSION

GLDH Activity and Vitamin C Content in Potato Tubers. GLDH activity on increasing GL concentrations revealed typical inhibition kinetics by high substrate concentration in the five potato cultivars assayed, which was in accordance with previous reports (**Figure 1**) (11, 16). However, inhibition by high substrate concentration has been reported to greatly depend on the plant source. For instance, GLDH from cauliflower required 32 mM GL to show substrate inhibition (16), whereas GLDH from potato required much lower GL concentrations, as shown in the present study and other reports as well (11). Nonlinear regression analysis of experimental data from **Figure 1** to eq 1 revealed that kinetic constants of the GLDH-catalyzed reaction also varied depending on the potato cultivar (**Table 1**). The greatest variation was observed for K_{SI} values, which ranged from 0.95 (Franceline potato) to 6.81 mM (Altesse potato). K_m values ranged from 0.23 (Monalisa potato) to 1.09 mM (Franceline potato). These values are in the range of those previously reported for GLDH from white potato (11).

In addition, it should be noted that GLDH activity from potato was lost after 3 days in both fresh (4 °C) and frozen (−20 °C) extracts. This behavior was in accordance with that reported for the stability of GLDH from spinach mitochondria, which became inactive after 15 h at 4 °C in 100 mM Tris-HCl (pH 8.0) containing 0.25 M sucrose (12).

The initial GLDH activity was compared with the corresponding initial AA content in each variety (**Table 1**) in order to investigate a possible simple correlation. However, a non-simple relationship was found between initial AA content and any kinetic constant that characterized GLDH activity from potato tuber (i.e., AA vs V_m, AA vs K_m, AA vs K_{SI}, and AA vs V_m/K_m) (results not shown). Therefore, initial AA content of potato tuber cannot be simply correlated to initial GLDH activity; other enzymes (phosphoglucose isomerase, phosphomannose isomerase, galactose dehydrogenase, etc.), and/or substrate precursors (fructose-6-phosphate, galactose, mannose-6-phosphate, GL) belonging to the AA biosynthesis pathway

Table 1. Kinetic Constants of GLDH[a] and Vitamin C[b] Content (AA+DHA) in Five Potato Tuber Cultivars (before cutting)

cultivar	V_m (μM/min)	K_m (mM)	K_{SI} (mM)	V_m/K_m (min^{-1})	AA (mg/100 g fw)	DHA (mg/100 g fw)	vitamin C (mg/100 g fw)
Manon	1.50 ± 0.08	0.41 ± 0.03	1.57 ± 0.13	$3.6 \times 10^{-3} \pm 3.5 \times 10^{-4}$	16.4 ± 0.7	3.2 ± 0.4	19.6 ± 2.5
Agata	1.85 ± 0.12	0.60 ± 0.04	1.63 ± 0.15	$3.1 \times 10^{-3} \pm 2.9 \times 10^{-4}$	6.1 ± 0.3	1.6 ± 0.2	7.7 ± 1.1
Monalisa	1.71 ± 0.11	0.23 ± 0.01	4.47 ± 0.41	$7.4 \times 10^{-3} \pm 6.8 \times 10^{-4}$	9.4 ± 0.3	3.2 ± 0.2	12.6 ± 0.9
Franceline	3.86 ± 0.25	1.09 ± 0.05	0.95 ± 0.08	$3.5 \times 10^{-3} \pm 2.8 \times 10^{-4}$	8.1 ± 0.3	2.2 ± 0.2	10.3 ± 1.1
Altesse	0.57 ± 0.03	0.42 ± 0.03	6.81 ± 0.51	$1.3 \times 10^{-3} \pm 1.1 \times 10^{-4}$	7.5 ± 0.2	1.8 ± 021	9.3 ± 1.1

[a] GLDH assay conditions are specified in Materials and Methods. Assay mixture included 60 μM Cyt *c*, 25 mM PB pH 8.0, 1 mM GL, 60 μM NaCN, and 20 μL of enzyme preparation (6 μg protein). [b] AA and DHA contents were determined by the method of Zapata and Dufour (*18*), with some modifications previously reported by Tudela et al. (*10*).

together with enzymes that regenerate ascorbic acid, such as dehydroascorbate reductase (DHAR) and/or monodehydroascorbate reductase (MDHAR), must be critically involved.

Effect of Fresh-Cutting and Storage Atmospheres on Vitamin C Content, GLDH Activity, and Browning. The potato cultivar Manon was chosen to investigate the effect of minimal processing and subsequent storage of potato strips in different controlled atmospheres (AIR, CA 20% CO_2, CA 100% N_2, and VAC) on vitamin C content and GLDH activity together with the influence on browning degree. This cultivar was chosen due to the combination of its intermediate GLDH activity and higher vitamin C content than the other cultivars (**Table 1**).

Vitamin C Content and Browning. Potato strips retained their vitamin C content after 6 days of storage in AIR at 4 °C (**Figure 2**), in accordance with a recent report (*10*). Although AIR storage is an interesting approach to monitor the overall process and even to preserve vitamin C content, obviously it is not a feasible way to keep potato strip quality, due to the severe browning development (**Figure 3**). Browning did not have relevant influence on vitamin C content in the present study, in agreement with a recent report which stressed the lack of correlation between browning development and vitamin C content (*25*). After 6 days of storage, vitamin C decreased in the rest of atmospheres following the sequence VAC (89% retention) > CA 100% N_2 (78%) > CA 20% CO_2 (63%) (**Figure 2**). The vitamin C content was approximately similar to that of AA in all treatments, because the quantitative variation of DHA after 6 days was minimal (**Figure 2**). According to our results, VAC packaging could be recommended for storing fresh-cut potatoes in the attempt to preserve both vitamin C content and browning degree (**Figures 2** and **3**). On the other hand, the storage of fresh-cut potatoes at high CO_2 atmosphere (CA 20% CO_2) yielded both the highest loss of vitamin C content and severe browning degree after 4 days (**Figures 2** and **3**).

GLDH Activity. GLDH activity of fresh-cut potatoes stored in different atmospheres was evaluated during the 6 day period to give a likely explanation to the observed vitamin C biosynthesis. Fresh-cutting provoked an increase of GLDH activity in potato strips in all the atmospheres reaching the maximum approximately at the fourth day of storage (**Figure 4**). The highest induction was observed in the strips stored in AIR (15.5-fold induction) and the lowest increase when stored in CA 20% CO_2 (6.7-fold induction). This meant that the overall tendency concerning the variation of vitamin C content during the 6 days could be explained, at least partially, by the corresponding GLDH activity (**Figures 2** and **4**).

It has been previously described that GLDH activity and AA content increased in both embryos and endosperm during the germination of *Pinus pinea* seeds. However, DHAR activity progressively decreased during germination, whereas MDHAR activity was the same in the endosperm and only slightly increased in the embryos. These results suggested that the increase of AA was mainly due to the novo synthesis, rather than to the recycling of the oxidized ascorbate (*26*). Therefore, these observations agree with our results and seems that the observed vitamin C retention after storage could be related with the induction of GLDH activity, and the increase in this enzyme could indicate high AA requirements during the storage period of fresh-cut potatoes. However, the full corroboration of this hypothesis should involve the determination of both DHAR and

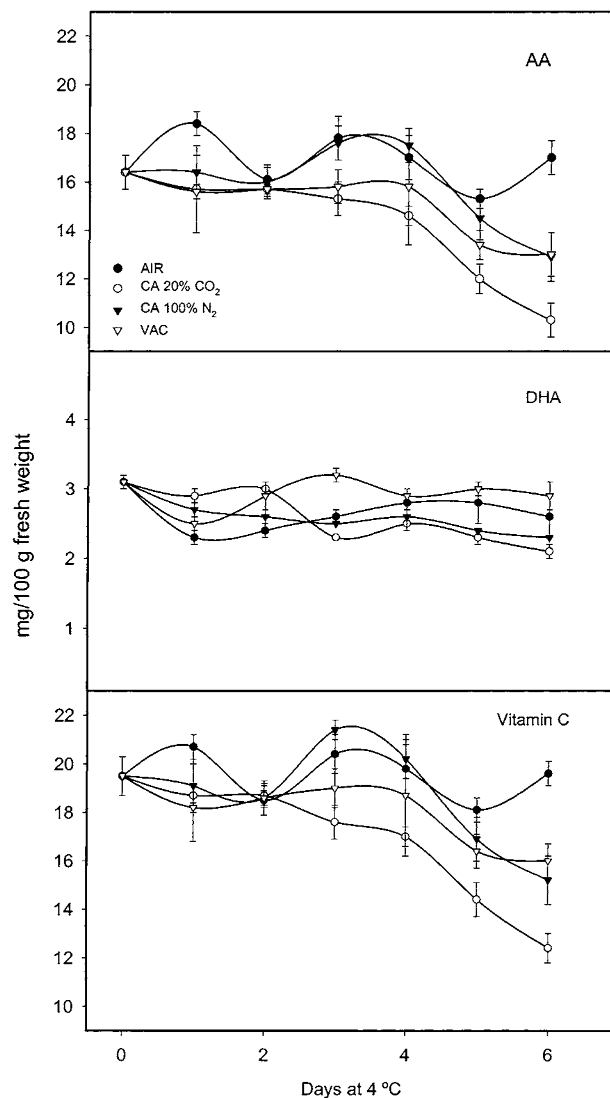

Figure 2. Influence of the atmosphere composition on the ascorbic acid (AA), dehydroascorbic acid (DHA) and vitamin C content during 6 days of storage at 4 °C of fresh-cut Manon potato strips.

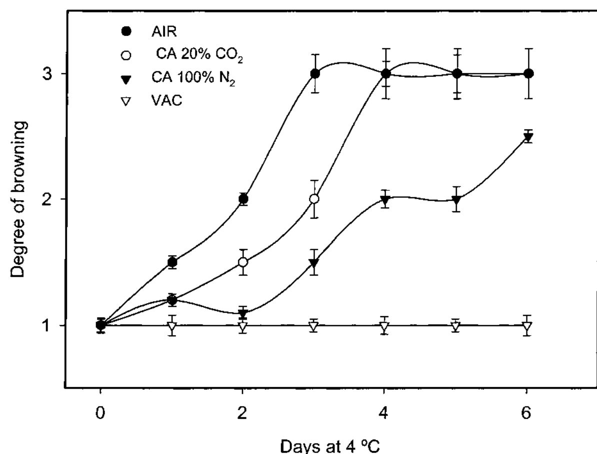

Figure 3. Effect of atmosphere composition on the degree of browning of fresh-cut Manon potato strips stored during 6 days at 4 °C. The scale ranged from 1 (no browning) to 3 (severe browning).

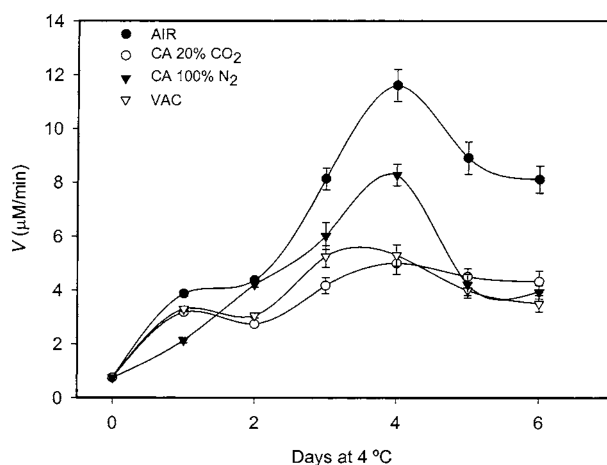

Figure 4. GLDH activity evolution of Manon potato strips stored during 6 days at 4 °C under different atmospheres. The reaction mixture contained 60 μM Cyt *c*, 25 mM PB pH 8.0, 60 μM NaCN, 1 mM GL, and 20 μL of enzyme preparation (6 μg protein) at 25 °C. Total assay volume was 1 mL.

MDHAR activities, which was not approached in the present study.

The highest AA retention (AA content at day 6 minus initial AA content) corresponded to the highest GLDH activity in AIR (GLDH activity at day 6 minus initial GLDH activity). On the other hand, the highest loss of AA was observed in the potato strips stored in CA 20% CO_2, which presented low GLDH activity (**Figures 2** and **4**). However, the daily variation of GLDH activity was not simply correlated with the daily evolution of AA, as demonstrated by the low correlation values (*R*) and high *P* values obtained (results not shown).

To deepen in the effect of fresh-cutting on GLDH, the kinetic behavior of this enzyme was evaluated every day in those potatoes strips stored in AIR. Storage in AIR was chosen because the GLDH activity was higher than that in the other atmospheres. **Figure 5A** shows the variation of GLDH kinetics versus days of storage. It should be stressed that the substrate inhibition kinetics observed in potato tubers (**Figures 1** and **5A**, day 0) changed to typical Michaelis kinetics, in which the inhibition by high substrate concentration disappeared (**Figure 5A**). To our knowledge, the change of GLDH kinetics as strategy to face wounding stress has not been previously published. In addition, apparent V_m values increased more than

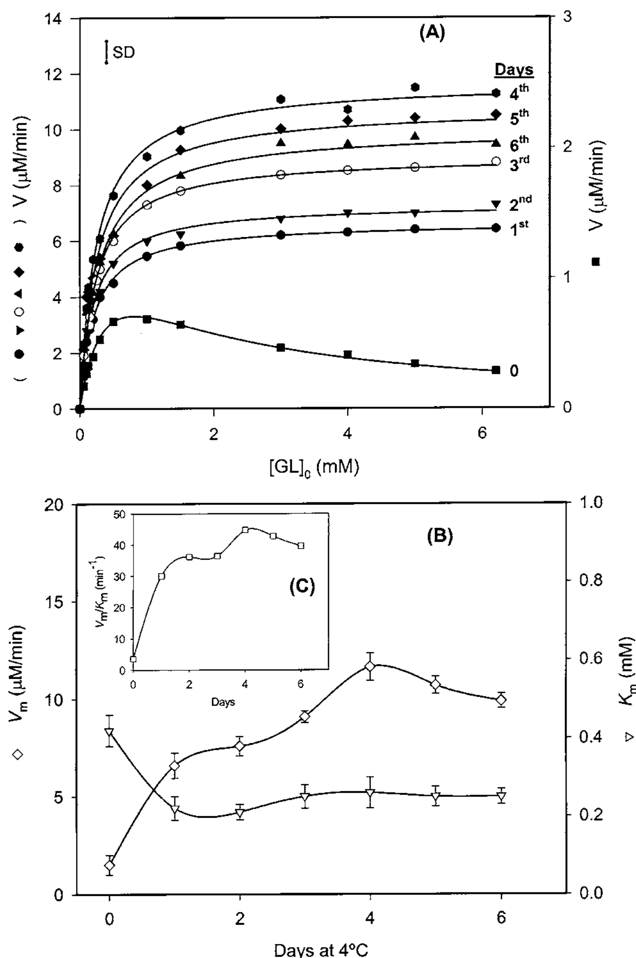

Figure 5. (A) Evolution of GLDH kinetics upon storing fresh-cut Manon potato in AIR during 6 days at 4 °C. (■) Initial GLDH activity in Manon potato tuber (before processing, day 0). Assay conditions were the same as those detailed in **Figure 4**. Symbols designate experimental data and solid lines the nonlinear regression fitting of experimental data to **eq 1** (in the case of potato tuber, ■) and to Michaelis equation ($v = V_m [S]/(K_m + [S])$ in Manon potato strips. **(B)** Evolution of V_m and K_m values (obtained from nonlinear regression analysis of **Figure 5A**) upon storing Manon potato strips in AIR during 6 days at 4 °C. **(C)** Evolution of GLDH catalytic power (V_m / K_m) (obtained from nonlinear regression analysis of **Figure 5A**) upon storing Manon potato strips in AIR during 6 days at 4 °C.

7-fold after 6 days of storage, whereas apparent K_m values decreased 2-fold the first day of storage after cutting and remained constant until day 6 (**Figure 5B**). The overall conclusion of this change is that the enzyme GLDH became more efficient to catalyze the conversion of GL to AA thanks to the 14-fold increase of the catalytic power (V_m/K_m) after 6 days of storage and the absence of inhibition by high substrate concentration from the first day after cutting (**Figure 5**). This could be related to the requirement of more antioxidant power by plant cells (by synthesizing AA) to face the wounding-generated stress by minimal processing (*17*, *27*). In addition, although other factors seem to be involved, this induction process was enhanced by the presence of oxygen and inhibited by high CO_2 concentration (**Figure 4**), which in turn, could be related to the lowest vitamin C retention observed in the strips stored under this atmosphere (**Figure 2**).

IEF experiments revealed that GLDH presented one major isoenzyme or isoform with isoelectric point 5 (IEP = 5) in all potato tuber cultivars before the processing (results not shown for all the cultivars, **Figure 6** A,B in Manon cultivar). The IEP

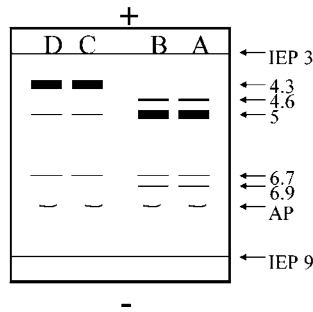

Figure 6. Isoelectric point (IEP) pattern of GLDH from Manon potato. Schematic drawing of the original gel (some faint bands were not visible after image processsing). The same pattern was obtained with the rest of potato cultivars (results not shown). **(A**, **B)**, Initial GLDH IEP pattern (day 0, before cutting). **(C)** GLDH IEP pattern after 1 day of storage at 4 °C in AIR. **(D)** GLDH IEP pattern after 6 days of storage at 4 °C in AIR. (AP, application point).

of the main isoform of the potato tubers assayed here is different to the IEP previously reported (IEP = 5.8) for GLDH from sweet potato roots (15). However, when the same experiment was carried out in fresh-cut Manon potatoes at different storage days, a shift of the major band was observed and the IEP changed from 5 to 4.3 (**Figure 6**, parts **C and D**). The same result was obtained for the rest of cultivars (results not shown). In addition, the bands with IEP = 4.6 and 6.9 at day 0 (**Figure 6**, parts A and B; before processing) disappeared after cutting and subsequent storage. It should be stressed that these changes were observed in only 1 day after fresh-cutting. Therefore, the change in the IEP pattern of GLDH gave a likely explanation to justify the change in GLDH kinetics upon fresh-cutting and subsequent cold storage. Whether the change in IEP pattern after minimal processing implied the induction of a new isoenzyme with IEP 4.3 or the change was due to postranslational modification from the isoform with IEP 5 to yield the new IEP 4.3 remains unanswered. According to the above results, this new GLDH isoenzyme or isoform was much more effective than that of potato tuber to catalyze the synthesis of AA from its GL precursor, which was mainly corroborated by the increase in the catalytic power (V_m / K_m) of the "new" enzyme (**Figure 5C**).

The present study is the first report concerning the evolution and kinetic characterization of the enzyme GLDH and its correlation with vitamin C content in fresh-cut potatoes. Fresh-cutting and subsequent cold storage (minimal processing) appears to trigger a cascade of events in potato tissue involving overlapped anabolic and catabolic reactions, such as the biosynthesis of antioxidant flavonols through the induction of the enzyme phenylalanine ammonia-lyase (*28*), the induction of oxidative enzymes, such as polyphenol oxidase and peroxidase (*25*), as well as the induction of vitamin C synthesis (*10*). Strategies addressed to retain vitamin C content in fresh-cut potates to keep their antioxidant potential should involve the preservation of the biosynthetic-vitamin C enzymes (such as GLDH or ascorbate-recycling enzymes such as DHAR and MDHAR), as well as the inhibition of catabolic-vitamin C enzymes with potential degradation of vitamin C (such as ascorbate oxidase). According to the results obtained, vacuum packaging should be recommended for keeping the sensory properties of potato strips together with a substantial retention of vitamin C content (89%).

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