

Lipoxygenase Pathway and Membrane Permeability and Composition during Storage of Potato Tubers (*Solanum tuberosum* L. cv Bintje and Désirée) in Different Conditions

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Abstract: Potato tubers (*Solanum tuberosum* L. cv Bintje and Désirée) were stored for 12 months under three different storage conditions: 4°C, 20°C with sprout inhibitor and 20°C without sprout inhibitor. Independent of the storage conditions, our results show that the increase of membrane permeability, as revealed by electrolyte leakage, is not correlated with the lipid saturation status. Moreover, there is no simple correlation between cold sweetening and membrane permeability or lipid saturation status. During storage at 20°C without sprout inhibitor, the increase in membrane permeability is inversely correlated to sucrose accumulation, but this is not the case when tubers were stored with sprout inhibitors. Lipoxygenase (LOX) is often proposed as responsible for peroxidative damage to membrane lipids. The gradual peroxidation resulting in double bond index decrease is regarded as a cause of senescence sweetening. Our results revealed that the role of LOX in aging and senescence of potato tubers is far from clear. LOX activity and gene expression are not correlated with the fatty acids composition of the membrane. Moreover, LOX activity and fatty acid hydroperoxide content are low in older tubers, whatever the storage conditions or the varieties. On the basis of our results, the correlation between sugar accumulation (low temperature and senescence sweetening) and peroxidative damage occurring during storage of potato tubers is discussed.

Key words: Fatty acid hydroperoxides, lipoxygenase, membrane integrity, potato tubers, *Solanum tuberosum* L., storage.

Abbreviations:

CIPC: isopropyl N-(3-chlorophenyl)carbamate
DBI: double bond index
GC: gas chromatography
LOX: lipoxygenase
MDA: malondialdehyde
ROOH: fatty acid hydroperoxides

Introduction

LOX (E.C. 1.13.11.12) is an intramolecular dioxygenase which catalyses the hydroperoxidation of polyunsaturated fatty acids containing 1(Z), 4(Z)-pentadiene structure. Depending on the origin of the LOX and on the reaction conditions, variable amounts of 13- and/or 9-hydroperoxides are formed (Siedow, 1991^[1]). Fatty acid hydroperoxides (ROOH) can be further degraded in a variety of products involved in major plant functions by different enzymatic systems: hydroperoxide lyase pathway (Delcarte et al., 2000^[2]), allene oxide synthase pathway, peroxygenase pathway (Blée, 1998^[3]). In some plants, including potato (*Solanum tuberosum* L.), a divinyl ether synthase pathway has also been described (Galliard and Phillips, 1972^[4]; Hoyaux et al., 2001^[5]). Expression of LOX genes is induced by different stress conditions: pathogen infection (Fidantsef and Bostock, 1998^[6]; Kolomiets et al., 2000^[7]), insect attack (Hildebrand et al., 1989^[8]), water deficit (Bell and Mullet, 1991^[9]) and wounding (Geerts et al., 1994^[10]; Royo et al., 1996^[11]). The enzyme is also involved in aging and senescence in various plant organs. A particular LOX working at acidic pH is expressed during senescence in rose petals (Fukuchi-Mizutani et al., 2000^[12]). LOX activity increases during senescence of day lily petals (Panavas and Rubinstein, 1998^[13]), carnation petals (Rouet-Mayer et al., 1992^[14]) and pea foliage (Grossman and Leshem, 1978^[15]). LOX is often proposed as responsible for peroxidative damage to membrane lipids during aging and senescence, however, its role is unclear. In soybean, LOX (I and II) activity decreases during aging of seeds (Sung and Chiu, 1995^[16]) or senescence of cotyledons (Peterman and Siedow, 1985^[17]). The same behaviour was observed in detached wheat and rye leaves (Kar and Feierabend, 1984^[18]). Although potato tubers provide a model system for studies of metabolic processes associated with aging and senescence, the information concerning LOX evolution during these processes is limited. As in many other plants, reducing sugars (glucose, fructose) resulting from starch breakdown accumulate in tubers under cold conditions. The amount of reducing sugars determines the processing potential of potatoes because they may react with amino acids during frying leading to an unacceptable dark colour (Van Kempen et al., 1996^[19]). Sweetening observed at higher temperatures after long-term storage is called senescence sweetening (Hertog et al., 1997^[20]). Sweetening has been explained by the peroxidative breakdown of amyloplast membranes leading to increased starch hydrolysis (Kumar and Knowles, 1993^[21]). A relationship between sugar accumulation

and membrane permeability has been demonstrated during cold storage of potato tubers (Spychalla and Desborough, 1990^[22]). Moreover, a negative correlation has been found with the double bond index (DBI) of fatty acids and electrolyte leakage (Knowles and Knowles, 1989^[23]). Peroxidative degradation of membranes is thought to be responsible for sweetening because a correlation was found between malondialdehyde (MDA), ethane and lipofuscin-like fluorescent compound content and reducing sugar accumulation (Kumar and Knowles, 1993^[21]). MDA, ethane and lipofuscin-like compounds are often presented as signs of lipid peroxidation but they are indirect markers of the oxidation process. In fact, a variable part of ROOH can undergo a cyclisation leading, after a non-enzymatic cleavage, to the formation of MDA. In addition, MDA can only be formed from fatty acids containing three or more double bonds (Frankel, 1991^[24]) and potato tubers contain high level of linoleic acid (C18:2) (Gravouille, 1996^[25]). Determination of MDA by the thiobarbituric acid assay is non-specific and subject to interference from many substances: pigments, aldehydes and particularly sucrose (Gray, 1985^[26]). Moreover, the thiobarbituric test is not correlated with fluorescence formation (Frankel, 1991^[24]).

In order to clarify the relationship between the phenomenon of cold and senescence sweetening with membrane metabolism, lipidic peroxidation was studied during storage of potato tubers by direct measurement of ROOH content and by determination of double bond index. The role of LOX was investigated by determining enzyme activity and gene expression during the storage process. Low temperature and senescent sweetening were followed by the determination of soluble sugar content (glucose, fructose, sucrose).

Materials and Methods

Plant material

Potato tubers (*Solanum tuberosum* L. cv Bintje and Désirée) were obtained at harvest from a local market. The tubers were measured and those between 60 and 65 mm were used for the study. The selected tubers were washed and stored for two weeks at room temperature to allow wound healing. The ages were all calculated from the end of the wound healing period when tubers were transferred to two distinct storage conditions. The storage conditions used were: 20°C, relative humidity 90% in the dark; 4°C, relative humidity 80% in the dark. The tubers stored at 20°C were separated in two batches: the first was treated with CIPC (20 g of a powder containing 1% [w/w] of CIPC per kg of tubers) to avoid sprouting while the second received no treatment and was allowed to sprout. The 4°C conditions and the 20°C conditions with CIPC treatment inhibited sprouting throughout the storage period. The tubers were stored for 12 months and samples were taken after 0, 15, 30, 45, 60, 120, 180, 240, 300 and 360 days. For the tubers stored at 20°C without sprout inhibitor, the sampling was stopped after 240 days because of the physical state of the tubers. For each variety and for each storage condition, 10 tubers were randomly sampled and cut transversely into two parts. In each tuber half, a core was taken with a punch (18 mm in diameter) perpendicular to the cutting axis. The cylinders obtained were recut from the opposite side of the skin to obtain 10 g. The samples were frozen in liquid nitrogen, powdered in a mill and stored at -80°C.

Sprout number

Potato tubers stored at 20°C without sprout inhibitor were observed visually to follow sprouting. When 80% of the tubers exhibited one or more sprouts, the potato stock was considered as sprouted. The results are the average of data from 30 tubers. For the tubers stored at 4°C, 10 tubers were transferred to 20°C to observe sprouting capacities at regular intervals. The results are averages of data from 10 tubers for each storage period at 4°C. Day zero corresponds to the transfer of the samples into the different storage conditions after the wound healing period.

Electrolyte leakage

The measurement of ion leakage from tuber tissue was based on the method of De Weerd et al. (1995^[27]). Values are the average of two determinations. The membrane integrity percentage was calculated as: % membrane integrity = $[1 - (\text{electrolyte leakage after 100 min of submersion} / \text{total electrolyte pool})] \times 100$.

Lipoxygenase activity measurement

Enzyme extraction: 10 ml of ice-cold sodium phosphate buffer (0.1 M, pH 7.5) containing sodium metabisulfite (2 mM) were added to 2 g of potato tuber powder. The extraction was conducted on ice under continuous stirring for 30 min. The mixture was centrifuged (4°C, 30 000 g, 30 min) and the supernatant was used directly as crude extract. Three independent extractions were performed on each sample.

Lipoxygenase assay: LOX activity was determined spectrophotometrically on the basis of absorbance increase at 234 nm due to appearance of conjugated diene in the ROOH formed. The measurements were performed on a UV-Vis spectrophotometer, Ultrospec 4000 from Pharmacia (Uppsala, Sweden), using linoleic acid as substrate. The LOX activity was expressed in Katal/kg protein. The data are the average of three repetitions.

Protein content in the crude extracts was determined spectrophotometrically according to Lowe and Thomas (1996^[28]).

Ratio between 13- and 9-linoleic hydroperoxides

Linoleic acid was added to a crude potato tuber enzymatic extract. Linoleic hydroperoxides formed were further analysed by HPLC according to Fauconnier et al. (1997^[29]).

Northern blot analysis

Lipoxygenase probe: cDNA fragments corresponding to *lox-1* were cloned by PCR from a λ ZAP swelling stolon cDNA library. *Lox-1* forward primer (5'-AGCACAGCAATCCAGCTAC-3') and *lox1* reverse primer (5'-GGTTCAGGCATGAATCTTCG-3'), designed according to a *lox1* sequence available in EMBL/GenBank (x95513), were used to clone a 2011 kbp fragment using the Topo TA cloning kit (Invitrogen). The authenticity of the sequence was verified by sequencing the PCR fragment using the ³²P Sequencing Kit (Amersham Pharmacia Biotech, Uppsala, Sweden).

RNA extraction and Northern blotting: Total RNA was purified from potato tissues using the method described by Kawakami et al. (1999^[30]). Twenty micrograms of total RNA were electrophoresed in a 1.2% agarose-formaldehyde gel and transferred onto a nylon membrane (Hybond-N, Amersham), as described by Sambrook et al. (1989^[31]). The membranes were prehybridized and hybridized in 0.5% SDS, 5 × Denhardt's solution, 6 × SSC, 200 µg/ml sonicated herring sperm DNA. Probes were ³²P-labelled DNA using a random primed DNA labelling kit (Amersham Pharmacia Biotech, Uppsala, Sweden). After hybridization, membranes were washed sequentially 2 × 10 min in 2 × SSC at room temperature, 2 × 30 min in 2 × SSC and 0.5% SDS at 65 °C, and 2 × 30 min in 0.1 × SSC at room temperature. The blot was exposed to X-ray films (X-Omat AR, Kodak) at -70 °C.

Sugar extraction and analysis

1 g of potato tuber powder was extracted in 10 ml of acetonitrile/water (70/30) for 1 h under continuous agitation. The mixture was centrifuged (10 000 g, 1 h, 4 °C) and the supernatant was used directly for HPLC analysis. 5 µl of extract were injected onto a 25 cm × 3 mm hypersyl 5 APS-2SS column (Chrompack, Middelburg, The Netherlands) with a flow rate of 0.7 ml/min. An Evaporative Light Scattering Detector (Alltech, Deerfield, IL, USA) with a tube temperature of 84 °C and a gas flow rate of 2.15 standard liters per min was used for the detection. Determination of the glucose, fructose and sucrose content was performed with calibration curves realized with different dilutions in acetonitrile/water (70/30) of glucose, fructose and sucrose (all 99% pure, Merck, Darmstadt, Germany). Two independent extractions were obtained for each sample and the sugar content was expressed in mg/g of fresh weight.

DBI

DBI was calculated after extraction and gas chromatography (GC) analysis of fatty acids, according to Lognay et al. (1988^[32]). GC analyses were realized on two independent extracts. For DBI calculation, the average of the two repetitions was considered and the formula of Wismer et al. (1998^[33]) was used: $DBI = [(2 \times \%C18:2) + (3 \times \%C18:3)] / [(\%C16:0) + (\%C18:0) + (\%C18:1)]$.

Fatty acid hydroperoxide content determination

Fatty acid hydroperoxides were determined spectrophotometrically based on their reaction with an excess of Fe²⁺ at low pH in the presence of xylenol orange, according to Griffiths et al. (2000^[34]). Data are averages of three repetitions performed on two independent extractions.

Statistical analysis of data

When the number of repetitions is above or equal to three, standard deviations were calculated and presented on the figures. When two repetitions were performed, residual standard deviations (average of standard deviations) were calculated and presented in the figure legends. Details on the sampling procedure are presented directly in each paragraph of the materials and methods section.

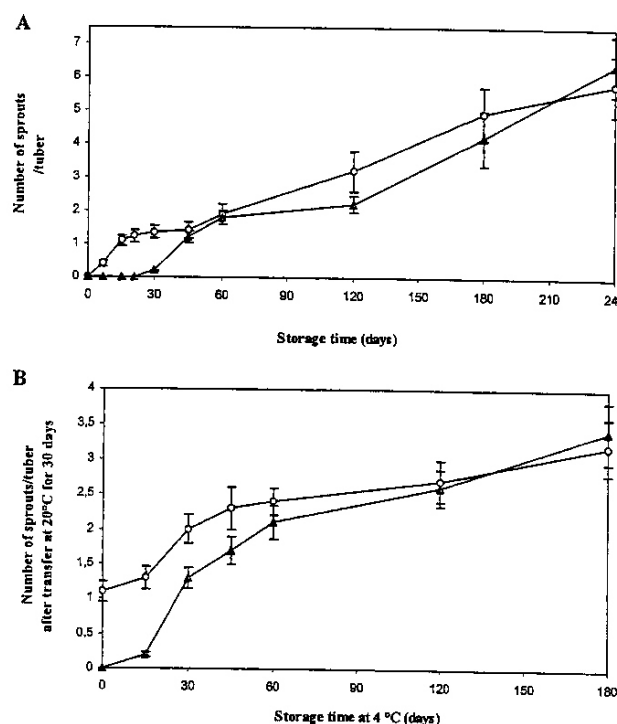


Fig. 1 (A) Sprout number per potato tuber during storage at 20 °C without sprout inhibitor, (○) Bintje, (▲) Désirée. The results are the average of data from 30 tubers, standard deviations are shown on the figure. (B) Sprout number per tuber of samples stored at 4 °C after transfer to 20 °C for 30 days, (○) Bintje, (▲) Désirée. The results are the average of data from 10 tubers, standard deviations are shown on the figure.

Results

Sprout number

During the storage of potato tubers, four stages of physiological development can be described (Ellisèche, 1996^[35]): (1) dormancy during which there is no sprouting even under favorable conditions, (2) apical dominance where only one sprout develops, (3) multiple sprouts stage where apical dominance is released and more than one bud sprouts at the same time, (4) daughter tuber stage where sprouts are replaced by daughter tubers appearing directly on the mother tuber.

The evolution of sprout number per tuber during storage at 20 °C is presented in Fig. 1A. On the basis of visual observations, the potato tubers stored at 20 °C without sprout inhibitor were considered sprouted after 15 and 45 days for cultivars Bintje and Désirée, respectively. The strong apical dominance lasts until around 60 days of storage. Between 60 and 240 days of storage, sprout number per tuber increases regularly. At the end of the storage period (240 days), the development of daughter tubers was observed. The latter observations are very similar for the two varieties.

Sprouting capacities of tubers stored at 4 °C were evaluated by regularly transferring samples to 20 °C and by observing the number of sprouts per tuber after 30 days (Fig. 1B). The sprouting phenotype can be considered as a marker of aging

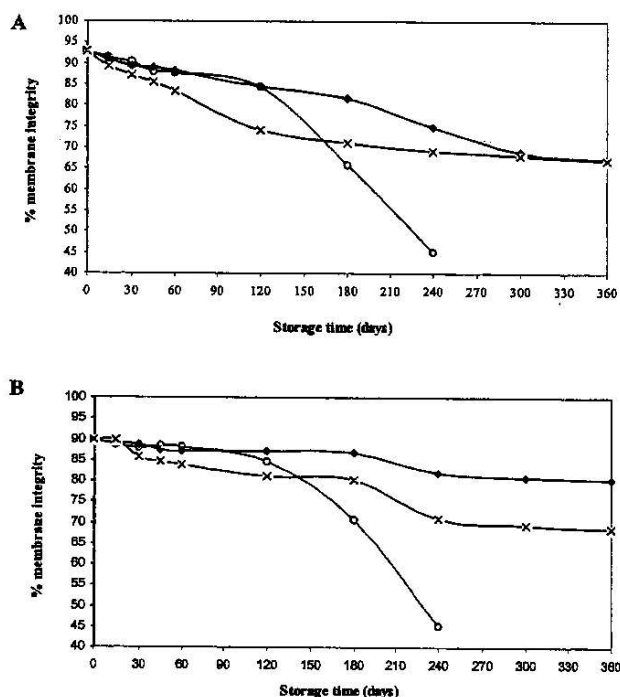


Fig. 2 Evolution of membrane integrity (electrolyte leakage measured after 100 min of submersion) during storage of potato tubers, (A) Bintje, (B) Désirée. (♦) Storage at 20°C with sprout inhibitor, (○) storage at 20°C without sprout inhibitor, (×) storage at 4°C. The results are the average of two independent determinations. Residual standard deviations are 0.294 (Bintje) and 0.311 (Désirée).

and senescence. Our results show that the four stages of physiological development described above are encountered in the samples stored at 4°C when they are transferred at 20°C, revealing that the aging and senescence phenomenon occurs during the 4°C storage. The number of sprouts per tuber after 30 days at 20°C increases as a function of storage time at 4°C due to a gradual loss in apical dominance. After 240 days of storage at 4°C, tubers transferred to 20°C begin to exhibit daughter tubers; after 360 days there were almost only daughter tubers and no more sprouts (not shown in Fig. 1B).

Electrolyte leakage

In our study, electrolyte leakage increases during potato tuber storage: at the beginning of the storage period, the reduction in membrane integrity percentage at 4°C is more important than that at 20°C. The evolution of membrane integrity percentage of tubers without sprout inhibitor (20°C) is similar to the tubers treated with sprout inhibitor (20°C) until 120 days of storage, then the membrane integrity percentage decreases dramatically for the sprouted samples (Figs. 2A,B). The evolution of membrane integrity percentage during storage of potato tubers revealed similar behaviour in the two varieties. Nevertheless, cultivar Désirée conserves a higher membrane integrity percentage than Bintje during the first six months of 4°C storage and throughout the 20°C storage with sprout inhibitor. The sprouting phenomenon is clearly not responsible by itself for the electrolyte leakage increase, but the role of sprout growth is a determinant as there is a marked differ-

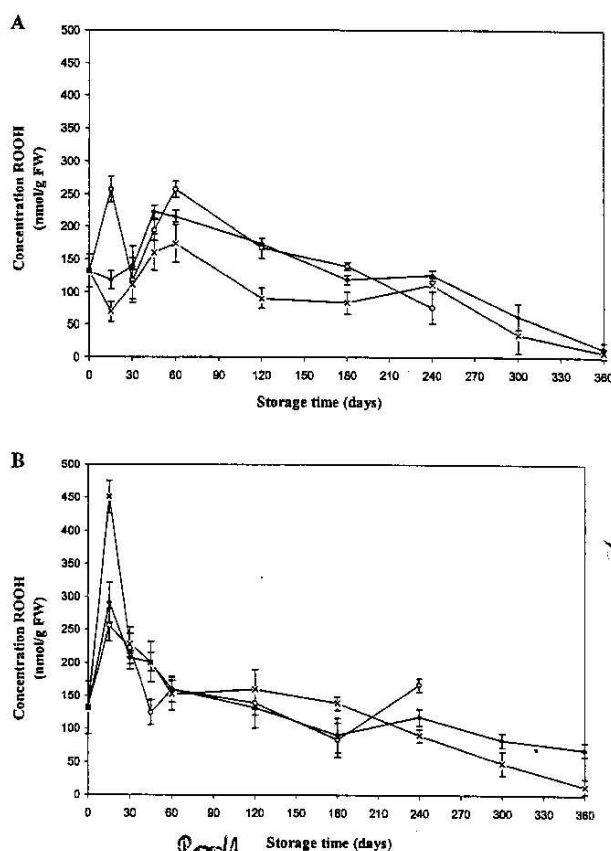


Fig. 3 Evolution of DBI during storage of potato tubers, (A) Bintje, (B) Désirée. (♦) Storage at 20°C with sprout inhibitor, (○) storage at 20°C without sprout inhibitor, (×) storage at 4°C. The results are the average of two independent determinations. Residual standard deviations are 0.351 (Bintje) and 0.322 (Désirée).

ence between samples stored at 20°C with or without sprout inhibitor. The role of desprouting to prevent electrolyte leakage has previously been reported by De Weerd et al. (1985).

Double bond index

DBI has a general tendency to decrease for both cultivars during the storage period but the three first months of storage are marked by important fluctuations (Figs. 3A,B). After one year of storage, DBI decrease is more important for tubers stored at 20°C with sprout inhibitor than at 4°C. Tubers stored at 20°C without sprout inhibitor show a DBI drop after 15 and 60 days of storage, respectively, for cultivar Désirée and Bintje, but after 240 days of storage, the DBI decrease is limited. DBI variations during storage may seem limited but as the total fatty acid composition of potato tubers reflects the composition of cell membranes (Spychalla and Desborough, 1990^[22]), slight modifications can result in important effects.

Lipoxygenase activity

LOX activity determination was performed on three independent extracts and three measurements were performed on each sample. LOX activity patterns during storage of potato tubers are similar for both varieties (Figs. 4A,B). LOX activity

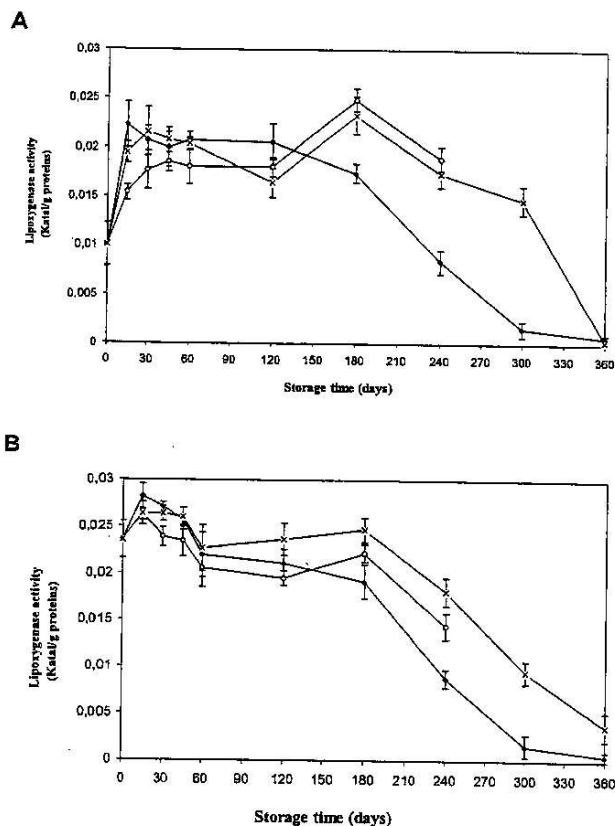


Fig. 4 Evolution of LOX activity during storage of potato tubers, (A) Bintje, (B) Désirée. (♦) Storage at 20°C with sprout inhibitor, (○) storage at 20°C without sprout inhibitor, (×) storage at 4°C. The results are the average of three independent determinations, standard deviations are shown on the figure.

increases substantially during the first 15 days of storage and then decreases to nearly zero after one year of storage. For samples stored at 20°C without sprout inhibitor and at 4°C, a peak of activity is observed around 180 days of storage. LOX activity is somewhat higher in Désirée than in Bintje and generally lower in samples stored at 20°C with sprout inhibitor. Decreasing LOX activity during aging has been described previously (Kar and Feierabend, 1984^[18]; Peterman and Siedow, 1985^[17]; Sung and Chiu, 1995^[16]) in different plant organs. During storage of potato tubers, different results have been reported: Lojkowska and Holubowska (1989^[36]) have observed important variations in LOX activity according to the varieties tested (6 varieties, 30 weeks of storage at $8 \pm 2^\circ\text{C}$) but showed a general tendency to increasing activities. Kumar and Knowles (1993^[21]) stored tubers from cultivar Russet Burbank at 4°C and transferred them after, respectively, 8 and 20 months into a greenhouse. LOX activity was determined polarographically in samples taken during the 30 days following planting. Lower LOX activity was systematically recorded in 20-month-old tubers than in 8-month-old tubers.

Northern blot analysis

Preliminary Northern blot experiments using *lox-1*, *lox-2* and *lox-3* probes showed that only *lox-1* can be detected in tubers stored at 4°C (data not shown). Further investigations were

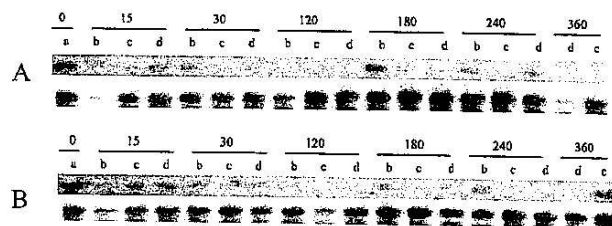


Fig. 5 Northern blot analyses of LOX (*lox-1*) gene expression during storage of potato tubers (cv. Désirée and Bintje). (A) Bintje, (B) Désirée. a: initial conditions, b: storage at 20°C without sprout inhibitor, c: storage at 4°C, d: storage at 20°C with sprout inhibitor. 0, 15, 30, 120, 180, 240, 360 days of storage.

performed with *lox-1* on a selected number of samples: RNAs of the two cultivars stored for 0, 15, 30, 120, 180, 240 and 360 days were extracted (Fig. 5). *Lox-1* gene expression was high in initial samples, decreased until 120 days of storage, increased again until 240 days of storage, and was almost undetectable after 360 days of storage, except for Désirée stored at 4°C. For cultivars Bintje and Désirée, *lox-1* gene expression was markedly higher in samples stored at 20°C, particularly for samples allowed to sprout, than at 4°C. Overall, *lox-1* gene expression follows the same tendency as LOX activity.

Ratio between 13- and 9-linoleic hydroperoxides

The ratio between 9- and 13-linoleic acid hydroperoxides formed by LOX extracts in the presence of linoleic acid was determined for samples of the two cultivars stored for 0, 30, 120, 240 and 360 days. The data are the average of two repetitions. The 9 isomer is almost exclusively formed by the extracts, whatever the cultivar or storage conditions: the ratio is between 98.9 and 99.6 for Bintje samples and between 98.9 and 99.9 for Désirée samples. In potato, three main isoforms of LOX have been described: LOX-1, found in tubers and roots, mainly forms fatty acid 9-hydroperoxides, while LOX-2 (leaves) and LOX-3 (leaves and roots) synthesize mainly fatty acid 13-hydroperoxides (Royo et al., 1996^[11]). According to our results, LOX-1 is almost exclusively present in tubers during the entire storage procedure for the two varieties, whatever the storage conditions.

Fatty acid hydroperoxide content determination

ROOH were determined spectrophotometrically by the Fox-2 method (Griffiths et al., 2000^[34]). The method yields very reproducible results but requires drastic precautions: all the experiments must be carried out in dim light, at 4°C with chilled solvents. ROOH concentrations found in our potato samples are lower than the values found by Griffiths et al. (2000^[34]) during the senescence of potato leaves, in which ROOH content increases from 334 to 611 nmol/g FW. On the other hand, our concentrations are above to those in *Phaseolus* hypocotyls and *Alstromeria* sepals or petals (Griffiths et al., 2000^[34]). The general pattern of ROOH content is characterized by an increase at the beginning of the storage period and a clear decrease afterwards (Figs. 6A,B). In Désirée samples, the increase is important and occurs after 15 days of storage, whatever the storage conditions, while the increase is less obvious in Bintje and occurs around 60 days of storage. High ROOH contents are expected in older samples but a similar decrease was observed

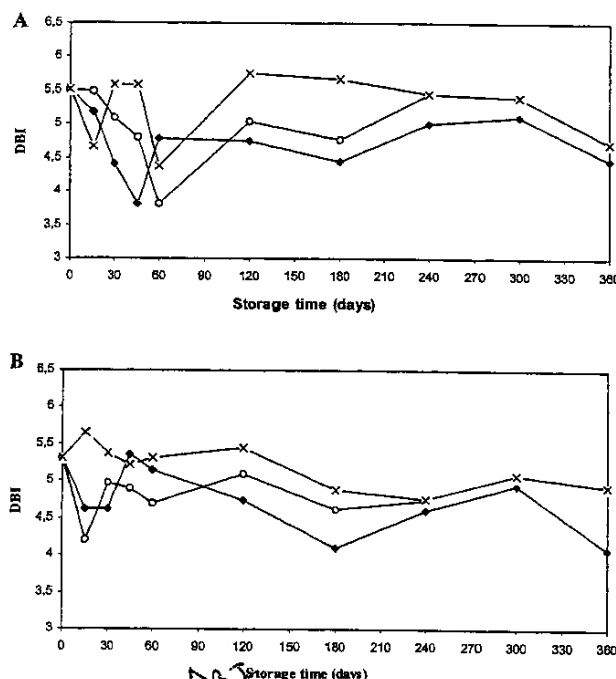


Fig. 6 Evolution of ROOH content during storage of potato tubers, (A) Bintje, (B) Désirée. (●) Storage at 20°C with sprout inhibitor, (○) storage at 20°C without sprout inhibitor, (x) storage at 4°C. The results are the average of three independent determinations, standard deviations are shown on the figure.

by Ye et al. (2000^[37]) in *Arabidopsis thaliana* leaf using the same Fox-2 method.

Sugar analysis

Potato tubers stored at 4°C accumulate soluble sugars, mainly the reducing sugars glucose and fructose. The ratio between fructose and glucose remains constant during the storage period, as described by Hertog et al. (1997^[20]). As expected, on the basis of their susceptibility to cold sweetening, sugar content is higher in cultivar Désirée than in cultivar Bintje at the end of a storage period at 4°C. In contrast, samples stored at 20°C with or without sprout inhibitor contain very low sugar concentrations until 120 days of storage; afterwards, the sucrose content increases almost linearly. In the latter samples, the accumulation of reducing sugars is low (Figs. 7A–F).

Discussion

Relation between electrolyte leakage, DBI and LOX

A high correlation ($r = -0.97$) was found between electrolyte leakage and DBI by Knowles and Knowles (1989^[23]) (cultivar Russet Burbank stored at 4°C during 32 months). Spychalla and Desborough (1990^[22]) found a less obvious correlation ($r = -0.47$) between electrolyte leakage and degree of fatty acid unsaturation for samples stored at 3°C; the correlation was not significant for samples stored at 9°C (4 different varieties stored 40 weeks without sprout inhibitor at 3°C, with sprout inhibitor at 9°C). Wismer et al. (1998^[33]) studied the early stage of storage (55 days at 4°C and 12°C, cultivars

Norchip and North Dakota) and observed fluctuations in DBI but no clear decrease and almost no evolution in electrolyte leakage. Our results reveal that no clear relationship can be established between electrolyte leakage and DBI, even if a general tendency to DBI decrease is observed for both varieties during the overall storage period. The most remarkable result is observed after day 120 for samples stored at 20°C without sprout inhibitor, when an increasing rate of membrane damage occurs, whereas no significant change in DBI is noticed. As LOX acts on C-18:2 and C-18:3 fatty acids, which are the main fatty acids in potato tubers, DBI is expected to be controlled by the LOX activity. Our results reveal that LOX activity is generally high during the first 180 days of storage, while DBI is almost unchanged and the ROOH content remains low. This last assumption suggests that potato tubers contain an efficient protection against ROOH accumulation that may cause membrane permeabilization when reinserted into the membrane and act as a calcium ionophore (Leshem, 1987^[38]). Enzymes that degrade ROOH may considerably influence the ROOH content, as shown by Griffiths et al. (2000^[34]) with potato leaves lacking hydroperoxide lyase that accumulate 38% more ROOH than the wild type. In potato tubers, hydroperoxide lyase is not a major enzyme but other ROOH degradation activities have been described. Divinyl ether synthase, that forms colneleic and colnelenic acid, two divinyl ether fatty acids, may be involved. However, our results (data not shown) reveal that only a part of the ROOH is transformed in colneleic acid and that no accumulation of this compound is observed during the storage period. Other enzymatic systems, such as glutathione transferase, may protect against ROOH accumulation. Kumar and Knowles (1996^[39]) have demonstrated a clear increase in glutathione transferase activity in tubers aged from 6 to 30 months (cultivar Russet Burbank stored at 4°C).

Overall, our data point out an erratic variation of DBI, LOX and ROOH content during the first two months of storage. A post-harvest stress may be implicated in the phenomenon but it does not result in a loss of membrane integrity. The role of LOX during germination and the early stages of sprout development requires additional investigations as the enzymatic activity and gene expression are high during these periods. We can postulate that the high ROOH content during this period can contribute to membrane remodelling. However, the relationship between LOX and germination is not obvious, as similar LOX activity profiles are observed for both varieties although sprouting kinetics are different. In the light of our results, it is necessary to identify the metabolic pathway responsible for membrane degradation, as LOX does not seem to be directly implicated; the main origin of electrolyte leakage is not the oxidation of unsaturated fatty acids. As the degradation of membrane lipids does not necessarily involve selective loss of unsaturated fatty acids (Mc Kersie and Leshem, 1994^[40]), oxygen free radicals or other mechanisms (e.g., hydrolytic pathways) may be responsible for electrolyte leakage without affecting DBI. Membrane proteins may also be degraded during the storage, decreasing membrane integrity.

Relation between electrolyte leakage and sugar accumulation

Low temperature sweetening and senescence sweetening result from different mechanisms and must be treated separately. Cold sweetening is a reversible mechanism since sugar content can be lowered by transferring tubers to higher tempera-

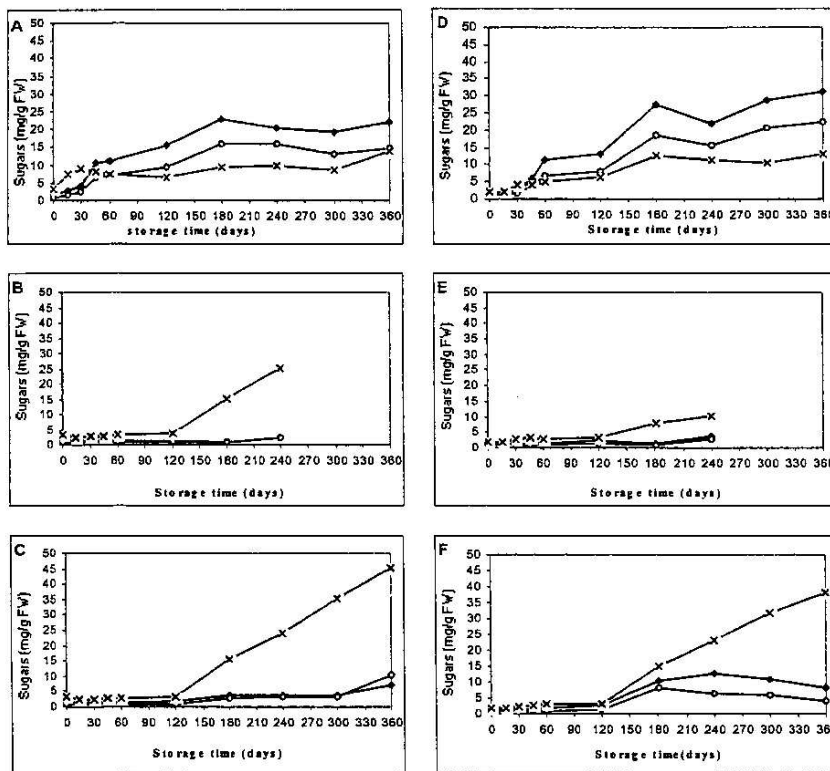


Fig. 7 Evolution of sugar contents (glucose, fructose, sucrose) during storage of potato tubers, (A) Bintje stored at 4°C, (B) Bintje stored at 20°C without sprout inhibitor, (C) Bintje stored at 20°C with sprout inhibitor, (D) Désirée stored at 4°C, (E) Désirée stored at 20°C without sprout inhibitor, (F) Désirée stored at 20°C with sprout inhibitor. (◆) glucose, (○) fructose, (×) sucrose. The results are the average of two independent determinations. Residual standard deviations are, respectively, (glucose, fructose, sucrose) equal to 2.5; 1.5; 1.0 (A) 0.2; 0.2; 1.1 (B) 0.4; 0.5; 2.6 (C) 3.1; 2.8; 1.4 (D) 0.6; 0.5; 0.9 (E) 1.0; 0.9; 2.4 (F).

tures (Van Kempen et al., 1996^[19]). Cold sweetening is a complex phenomenon, certainly not controlled by a single factor (Sowokinos, 1990^[41]). Moreover, it must be considered in a dynamic way, as exemplified by Hill et al. (1996^[42]) using ¹⁴C glucose labelling, sugars being simultaneously synthesized and remobilized. Membrane damage resulting in electrolyte leakage is often presented as the cause of cold sweetening. Spy-challa and Desborough (1990^[22]) have shown a correlation ($r=0.83$) between electrolyte leakage and reducing sugar accumulation (four different varieties stored for 40 weeks without sprout inhibitor at 3°C, with sprout inhibitor at 9°C).

Peroxidative degradation of membranes is particularly pointed out in the cold sweetening process, as a correlation was found between MDA, ethane and lipofuscin-like fluorescent compounds content and reducing sugar accumulation (Kumar and Knowles, 1993^[21]). The methods used to determine membrane peroxidation are indirect methods that can be criticized. As stated above, only fatty acids containing three or more double bonds can be transformed in MDA, while linoleic acid is much more abundant than linolenic acid in tubers (Frankel, 1991^[24]). Moreover, only a part of linolenic acid hydroperoxide will be transformed in MDA. Finally, as there is an interference from sucrose, which accumulates during storage, in the MDA determination method, the correlation between the two parameters seems somehow questionable. Our results reveal that the accumulation of reducing sugars is observed from the beginning of the storage period at 4°C, but not at 20°C (with or without sprout inhibitor). As no clear difference in LOX activity was noted when comparing the treatments, this enzyme should not be a major contributor to the cold sweetening process. However, loss of membrane integrity was slightly more rapid

at 4°C than at 20°C at the beginning of storage, which could be relevant for sugar metabolism.

The reversibility of cold sweetening is not in agreement with the hypothesis of lipidic peroxidation of membranes. It can be added that transgenic potatoes, presenting increased recycling activity of sugar into starch (ADP glucose pyrophosphorylase), tend to show lower cold sweetening susceptibility and respond more effectively to a subsequent increase in temperature by lowering their sugar content, which demonstrates that membranes are not irrevocably degraded (Davies et al., 1998^[43]).

Senescence sweetening is a non-reversible mechanism that occurs after long-term storage (Van Kempen et al., 1996^[19]). The relationship between senescence sweetening and membrane damage is clearly invalidated by our results: at 20°C, sucrose accumulates in the same way in samples showing a high degree of membrane degradation (sprouted tubers characterized by a decrease in membrane integrity percentage and by a loss of apical dominance, or by the presence of daughter tuber), or limited damage (tubers treated with anti-sprouting agent).

Sprouting, a major event in tuber physiology, is neither correlated with sugar accumulation nor with membrane integrity decrease, but sprout development clearly influences membrane integrity. The accumulation of sucrose is caused by a phenomenon not correlated with sprouting or membrane damage and requires further investigations to shed light on the mechanism.

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