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Identification of potato varieties suitable for cold storage and reconditioning: A safer alternative to anti-sprouting chemicals for potato sprouting control

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ABSTRACT

Low temperature storage as an alternative to anti-sprouting chemicals in potato storage may induce reducing sugars (RS) accumulation (*i.e.* glucose and fructose) in potato tubers. This phenomenon is called "cold induced sweetening" (CIS) and occurs in certain varieties. CIS leads to a decrease in the organoleptic qualities and darkening of processed potato and the accumulation of toxic molecules such as acrylamide. To identify potato varieties suitable for storage at low temperatures, we screened six commercial processing varieties: Lady Claire (LC), Verdi, Kiebitz (KB), Pirol, Agria and Markies for their CIS characteristics and sprout-forming potential after storage at 4 °C and 8 °C. Our findings reveal that 4 °C storage allows for efficient sprout reduction in all six tested varieties for up to 4.5 months of storage. Three CIS-resistant varieties, namely Verdi, Lady Claire and Kiebitz, were identified as able to be stored for up to four months at 4 °C with limited increase in glucose content. Conversely, Pirol, Agria and Markies showed an increase in glucose content with a decrease in storage temperature and can be considered as CIS-susceptible varieties. After processing into crisps, the CIS-susceptible varieties displayed poor crisp color quality (brown to black color crisps) after storage for two months at 4 °C compared to the storage at 8 °C, whereas the CIS-resistant varieties had good crisp color quality (pale yellow color crisps) after storage at both 4 and 8 °C. Interestingly, the trends of total RS and/or glucose content in the CIS-resistant and in the CIS-susceptible varieties were correlated with the trends in *Vacuolar Invertase* (*VInv*) gene expression for most varieties, as well as with the trends in acrylamide content after processing. In addition, reconditioning of Markies variety after storage at 4 °C by gradually increasing the temperature to 15 °C resulted in a significant decrease of *VInv* transcript levels (reduction of 80 %), acrylamide content (reduction of 75 %) and glucose content when compared to a storage at 4 °C without reconditioning. Those results demonstrate that the reconditioning technique is a key factor for a sustainable potato storage and for improving the quality of processed potatoes.

1. Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important food crop worldwide in terms of annual production with 370 million tonnes in 2019 (Faostat, 2021). To avoid losses caused by sprouting during potato storage, chlorpropham, commonly called CIPC, has been used for decades as the anti-sprouting agent of choice in the EU and Switzerland and is highly efficient to keep potatoes free of sprouts for months at

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storage temperatures ranging from 8 to 12 °C (Corsini, Stallknecht, & Sparks, 1979; Mahajan, Dhatt, Sandhu, & Garg, 2008). Nevertheless, the European Union recently voted for the non-renewal of the use of CIPC in potato storage due to a potential risk of CIPC and its metabolite 3-chloroaniline for the consumer (EU regulation 2019/989). Alternative treatments controlling sprouting are available including both natural and chemical molecules. For instance, studies have demonstrated the efficacy of essential oils to control sprouting such as orange oil (D-limonene) (Visse-Mansiaux et al., 2020) and mint oil (L-carvone) (Bruno et al., 2023; Şanlı & Karadoğan, 2019; Visse-Mansiaux et al., 2020; Vokou, Vareltzidou, & Katinakis, 1993). However, these molecules are often less effective than CIPC (Visse-Mansiaux et al., 2021; Visse-Mansiaux et al., 2020) and residues of treatments could persist in tubers. Visse-Mansiaux et al. (2021) reported that residues of the maleic hydrazide anti-sprouting molecule have been detected in tubers at the end of the storage period, even though these residues were below the authorized maximum residue limit (MRL).

Other storage strategies can be used to keep potatoes free of sprouts without relying on anti-sprouting molecules such as using varieties with long dormancies as the dormancy period varies according to the variety (Visse-Mansiaux et al., 2022). The definition of potato dormancy is subject to discussion in the literature. Reust (1986) defined dormancy as a physiological state of the tuber that does not allow it to germinate, even if tubers are placed under ideal conditions for sprout growth, while for Van Ittersum and Scholte (1992) the dormancy is the period from haulm killing to the stage with 80 % of sprouted tubers. However, it is clear that potato sprouting during storage occurs after the break of dormancy (Coleman, 1987; Daniels-Lake & Prange, 2007; Delaplace, Brostaux, Fauconnier, & du Jardin, 2008; Visse-Mansiaux et al., 2022).

However, because of market and agricultural requirements, varieties with short- and medium- dormancies are frequently used (Curty, Personal communication). To use varieties with a large range of dormancies while avoiding the use of chemicals to control sprouting, storage at low temperatures can be used to prolong the dormancy period and to delay sprouting (Burton & Wilson, 1978; Gichohi & Pritchard, 1995; Paul, Ezekiel, & Pandey, 2016; Sonnewald, 2001; Sowokinos, 2001). Potatoes intended for the fresh market are usually stored at low temperatures ranging from 5 to 6 °C (Bishop, Rees, Cheema, Harper, & Stroud, 2012). Storage at low temperature is generally not recommended for potato varieties dedicated for processing. Due to their susceptibility to sweetening, varieties dedicated for processing are usually stored at temperatures ranging from 7 to 10 °C (Bishop, Rees, Cheema, Harper, & Stroud, 2012). Sweetening of tubers at low temperatures is caused by the accumulation of reducing sugars (RS) (*i.e.* glucose and fructose), also called "cold-induced sweetening" (CIS) (Hou et al., 2017; Sowokinos, 2001) and controlled by complex mechanisms (Datir et al., 2020). During storage at low temperatures, potato starch is degraded mainly through phosphorylase in hexose-phosphates (Davis & Viola, 1992; Hill, Reimholz, SchrÖDer, Nielsen, & Stitt, 1996; Morrell & Rees, 1986; Sowokinos, 2001) leading to the accumulation of free sucrose (Sowokinos, 1990; Sowokinos, 2001). Sucrose is then cleaved by acid invertase to RS (McKenzie, Chen, Harris, Ashworth, & Brummell, 2013). During the CIS phenomenon in potato tubers, the rate of conversion of starch into RS is accelerated through several enzymatic reactions (Sowokinos, 2001). CIS leads to a decrease in quality of processed potatoes (*e.g.* crisps and French fries) and is partly responsible for bitter-tasting and dark coloration of crisps and French fries, which is unacceptable to consumers (Amjad, Javed, Hameed, Hussain, & Ismail, 2020; Mottram, Wedzicha, & Dodson, 2002; Pinhero, Pazhekattu, Marangoni, Liu, & Yada, 2011). CIS also leads to the production of toxic compounds such as acrylamide during frying, which may raise concerns for human health (Paul et al., 2016; Wiberley-Bradford & Bethke, 2017; Wiberley-Bradford, Busse, & Bethke, 2016) as acrylamide is classified as a probable human carcinogen (FAO/WHO, 2002). Undesirable flavor, dark color and acrylamide are produced through the Maillard reaction, that occurs during heat treatment and consists of a reaction between amino acids and RS. Asparagine has been demonstrated to be a major amino acid involved in the production of acrylamide in potatoes (Mottram et al., 2002).

Two main metabolic pathways control the CIS phenomenon: the sucrose synthesis controlled by several enzymes and the hydrolysis of sucrose to reducing sugars mainly controlled by the level of acid invertase activity (Pressey & Shaw, 1966; Sowokinos, 2001; Stitt & Sonnewald, 1995). The *vacuolar invertase* (*VInv*) gene seems to play an important role in potato CIS. Bhaskar et al. (2010) demonstrated that silencing this gene prevents the accumulation of RS in potatoes stored at low temperature and that the transcript levels of *VInv* were low in tubers resistant to CIS. At low temperatures, sucrose concentrations increase and sucrose becomes the substrate for *vacuolar invertase* leading to accumulation of RS (Duplessis, Marangoni, & Yada, 1996).

In the context of pesticide reduction, storing potatoes at low temperature to avoid sprouting instead of using chemicals seems to be a good practice to promote. To avoid CIS and human health risks associated with the presence of acrylamide in processed potatoes, potato varieties with limited accumulation of RS at low temperature (CIS-resistant varieties) should be used for cold storage. A few varieties with low accumulation of reducing sugars at low temperatures are available such as White Pearl (Groza et al., 2006), Sempra or Verdi (Böhm et al., 2006). With the CIPC non-renewal, it would be of interest to have a larger panel of CIS-resistant varieties, which can be stored at low temperatures. Therefore, it is necessary to describe the CIS-characteristics of several varieties before considering storage at low temperature and proposing CIS-resistant varieties with large ranges of dormancy.

Another strategy commonly used by the industry is to use cold storage followed by warming the tubers for one to several weeks at temperatures between 12 and 15 °C before processing. This practice allows the RS content to decrease and is also called "reconditioning" (Fitzpatrick & Porter, 1966; Jansky & Fajardo, 2014; Schippers, 1975).

Reconditioning could be used to reduce RS in CIS-susceptible varieties, improve the quality of crisps and French fries after cold storage, and consequently decrease the risk of acrylamide formation in deep-fried products. For instance, Poberežny, Wszelaczyńska, Gościnna, and Spychaj-Fabisiak (2021) studied the effect of a reconditioning and report an improvement of French frie quality made from tubers stored at low temperature with a reconditioning period. However, the reconditioning potential to decrease RS has been reported to be genotype-dependent (Blenkinsop, Copp, Yada, & Marangoni, 2002; Kyriacou, Siomos, Ioannides, & Gerasopoulos, 2009). Consequently, the potential of reconditioning should be evaluated for different potato varieties.

The main objectives of the present study are to identify potato varieties adapted to storage at low temperatures in relation to CIS, to evaluate the efficacy of reconditioning to reduce the RS content in potatoes after storage at low temperature, and to characterize genetic and enzymatic mechanisms involved in CIS.

For this research, six processing potato varieties, namely Verdi, Lady Claire (LC), Kiebitz (KB), Pirol, Agria and Markies were stored at low temperature (*i.e.* 4 °C) and the commonly used temperature (*i.e.* 8 °C). To understand the interaction between variety and storage temperature on potato tuber quality, several parameters such as tuber dormancy, sugar composition, asparagine content and *VInv* gene expression in tubers, as well as crisp color and acrylamide content in crisps were determined. In addition, the potential of reconditioning at 15 °C after storage at 4 °C was evaluated in tubers of the varieties Markies and Verdi.

2. Materials and methods

2.1. Plant material and experimental design

The six potato varieties: LC, Verdi, KB, Pirol, Agria and Markies were planted in April, harvested at the end of August, or beginning of September in Reckenholz (ZH, Switzerland) and stored for three seasons

(2016–2017, 2017–2018 and 2018–2019). These varieties have been selected because they are becoming of interest to the industry which tries to mitigate the problem of CIS during long-term storage. Upon harvest, tubers were stored at room temperature (about 15 °C) for two weeks to promote healing and afterwards storage trials were performed at two sites: Changins (VD, Switzerland) for two storage seasons 2017–2018 and 2018–2019, and Reckenholz for the three seasons: 2016–2017, 2017–2018 and 2018–2019. At each site, half of the tubers of each potato variety were stored in two different chambers at 12 °C and 85–90 % RH until the end of October or beginning of November. Then, the temperature was gradually decreased to 4 °C for the first chamber and to 8 °C for the second chamber with a decrease of 1.5 °C per week. Both final temperatures were reached at the end of November, date of the beginning of the storage. Tubers were stored at both temperatures until mid-April. In addition, for some of the tubers of Markies and Verdi varieties stored at 4 °C, a reconditioning treatment was applied from 4 to 15 °C with an increase of 1.5 °C per week to reach 15 °C after four months of storage (at the end of March). Tubers of the different tested varieties were sampled in chambers at 4 °C, 8 °C or after reconditioning at 15 °C at different periods to perform several measurements described in the sections below.

The storage trials described above were conducted with tubers from the same batch, all grown at the "Reckenholz" site. The tubers stored at 8 °C for four months at the Reckenholz site were treated with CIPC while tubers stored at 4 °C (with or without reconditioning) were untreated. Tubers stored at the Changins site at 8 °C and 4 °C remained untreated (with or without reconditioning). This experiment follows a split plot experimental design with two fixed effects: temperature and variety and was repeated over two to three seasons of storage.

2.2. Measurements performed on the stored potatoes

2.2.1. Physiological measurement: Sprouting evaluation

Sprouting of potatoes was evaluated on tubers stored in the "Changins" site, after 4.5 months of storage at 4 and 8 °C (until Mid-April) for the six potato varieties. Sprouting evaluation was performed by measuring the total weight of sprouts for one year of testing (2018–2019 season) and on four replicates of five tubers each and for each variety and temperature (sum of the weight of sprouts from five tubers, measurements performed on sprouts with a minimum size of 1 mm). Data of sprouting observation were analyzed using a two-way Anova. The fixed effects were the variety and the temperature. Sprouting variable was transformed to "log (x + 1)" to ensure the homogeneity of the variance and normality of the response variable. Significance test was performed with the F test provided by the "car" R package, version 3.0–7 (Fox & Weisberg, 2019). To analyze the effect of significant variables, the marginal post hoc Tukey's test (emmeans method) was used as a multiple comparison test to identify mean differences within factors and interactions (Lenth, 2020).

2.2.2. Measurements after processing: Frying test and acrylamide content

Frying color and acrylamide content were estimated on samples stored in the "Reckenholz" site during three seasons (2016–2017, 2017–2018 and 2018–2019). For each sample, 10 tubers were peeled with a peeling machine (model: Mini-Flott 35, Flott, Rotenburg an der Fulda, Germany). Tubers were washed in tap water for 30 to 60 s to remove the starch. For each tuber, a slice of 1.2 mm was cut at the center of 10 tubers to make the crisps using a cutting machine (professional machine, Art.–No.: 50,003 from https://www.patate.ch, Berne, Switzerland). Slices were fried for three minutes at 170 °C using a professional fryer (model: EVO 400 T, Valentine®, Romanel-sur-Morges, Switzerland) in high oleic sunflower oil (Florin AG, Muttenz, Switzerland). The 10 crisps per sample were placed in a white paper and color evaluation was performed visually using a scale ranging from four (=crisps fully dark) to seven (=crisps clear) (refer to the appendix). In general, a score of seven is considered as an acceptable result and a score from four to six is usually not acceptable for the potato processing industry. We adjusted a two-way binomial generalized linear mixed model on the number of crisps with acceptable color (note = seven) among the 10 tested crisps using the "lme4" R package version 1.1–23 (Bates, Maechler, Bolker, & Walker, 2015). The fixed effects of this analysis were the variety and the temperature, and the year was considered as the random factor. The effect of the year was removed from the model when the variance of the year was not significant.

We generated the Anova table corresponding to our model using the "car" R package, version 3.0–7, and using the chi-square significance test or the F test in cases where the effect of the random factor was not significant (Fox & Weisberg, 2019). Analyses were performed separately for the different periods: after two months of storage on six varieties and two storage temperatures (*i.e.* at the end of January at 4 and 8 °C) (N = 3 years); after four months of storage on six varieties and two storage temperatures (*i.e.* at the end of March at 4 and 8 °C) (N = 2 years); and after four months of storage in two varieties and three temperature regimes (*i.e.* at the end of March at 4 °C, 8 °C and 4 °C with a reconditioning to reach 15 °C at four months of storage) (N = 2 years). Tukey's post-hoc test (emmeans method) was performed to decompose significant fixed effects (Lenth, 2020). For the sake of conciseness, in cases of significant interaction between factors variety and temperature, the Tukey's test was performed directly on the interaction.

Acrylamide content analyses were performed on crisp samples for the six varieties obtained from the season of testing 2018–2019 after four months of storage at 4 and 8 °C, and after four months of storage with a reconditioning from 4 to 15 °C on two potato varieties (Verdi and Markies). Crisp samples (10 crisps per sample) were stored in double sealed plastic bags in the freezer at −20 °C for one month before the analysis. Before acrylamide extraction, the samples were crushed and ground to a powdery material with a rolling pin. After homogenization, 4 g of each sample were extracted with ultrasonic treatment for 30 min at 60 °C in duplicate by addition of 50 mL distilled water and 50 mL of deuterated acrylamide as internal standard, 150 μg mL^{-1} d3-acrylamide solved in ethyl acetate (acrylamide-2,3,3-d3, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Extracts were centrifuged for 5 min at 5,000 g and the supernatants were defatted using petroleum ether. For clarifying, Carrez I solution (potassium hexacyanoferrate (II) trihydrate, 15 %) and Carrez II solution (zinc sulphate, 30 %), 5 mL each, were added and extracts were centrifuged again at 5,000 g for 5 min. After subsequent salting-out (sodium chloride), acrylamide was extracted with 30 mL ethyl acetate three times from the aqueous phase. Water was separated from the organic phase of the acrylamide extract by a water-repellent filter (MN 616 wa ¼; Macherey-Nagel, Düren, Germany), followed by an evaporation to an approximate 0.5 mL residue. GC–MS analysis on a Trace GC/Trace DSQ II instrument (Thermo Scientific, Dreieich, Germany) was performed in the chemical ionization mode (reagent gas: methane at a flow rate of 1.3 mL/min) by the use of a Restek Stabilwax Integra-guard column (Restek GmbH, Bad Homburg, Germany, 30 m x 0.25 mm i.d., 0.25 μm film thickness). Separation was performed with a flow rate of 1 mL min^{-1} and helium was used as the carrier gas. After 2 min constant conditions at 60 °C, the column temperature was increased linearly to 240 °C with 20 °C per min and was finally held at 240 °C for 11 min. Ion source temperature and interface temperature were 180 °C and 240 °C, respectively, furthermore a split/splitless injector was used (splitless, temperature 200 °C). Detection was carried out in selected ion monitoring mode with m/z values of 72 (acrylamide) and 75 (d3-acrylamide). The internal deuterated acrylamide standard was used for quantification, and the limit of detection (LOD) was 10 μg kg^{-1} and limit of quantification (LOQ) was 20 μg kg^{-1}. Detailed description of the extraction and measurement is given by Haase, Grothe, Matthäus, Vosmann, and Lindhauer (2012).

2.2.3. Biochemical measurements: Sugar analysis

All biochemical measurements were performed on tubers stored in the "Changins" site.

To analyze sugar content (glucose, total RS and sucrose) in potatoes, five biological replicates of three tubers were sampled for each variety, temperature of storage and observation period (*i.e.* after two months = end of January, and four months of storage = end of March) over two years of testing (tubers from the seasons 2017–2018 and 2018–2019).

Glucose analysis was performed on fresh tubers using a glucose titer measurement device (Accu-Chek® Active glucometer, Roche, Mannheim, Germany) and with at least two analytical glucose measurements per tuber. We used the protocol of Olsen, Woodell, and Frazier (2011) with some modifications. For one analytical measurement, the potato was cut in two parts; a knife was used to macerate the tissue at the center of one potato part to obtain enough juice. Then, a test strip (suitable for this glucometer) was introduced in the glucometer, and a drop of juice was sampled using a 200 μL micropipette and placed on the test strip to obtain the measurement. The glucometer allows measurements ranging from 10 mg dL^{-1} to 600 mg dL^{-1} (0.6 mmol/L – 33.3 mmol/L) and outside this interval, the device indicates "low" or "high". Olsen, Woodell, and Frazier (2011) reported that, in the more stringent cases, glucose levels of 0.05 % on a fresh weight (fwt) basis are required in tubers before processing to avoid unacceptable fry color. In their study, glucose level of 0.05 % fwt corresponded to values of 75 mg dL^{-1} with the glucometer. Based on these results, we considered glucose values greater than 75 mg dL^{-1} as unacceptable for processing and glucose values $\leq$ 75 mg dL^{-1} as acceptable for processing. Consequently, the raw data were transformed into binary categories: 0 = acceptable sugar content in potatoes ($\leq$75 mg dL^{-1}) and 1 = unacceptable sugar content in potatoes (>75 mg dL^{-1}). The percentages of acceptable samples for each variety and temperature over two years were calculated and asymmetrical confidence level estimated by the specific formula of the Agresti-Coull function in R (lower or upper Agresti-Coull confidence limits) appropriate for the analysis of binary data was added to the graphics using the "binom" R package version 1.1–1 (Agresti & Coull, 1998; Dorai-Raj, 2014).

Analysis of glucose content results was performed separately for the different periods: after two months of storage (end of January) for six varieties and two storage temperatures (4 and 8 °C); after four months of storage (end of March) for six varieties and two storage temperatures (4 and 8 °C); and after four months of storage for two varieties and three temperature regimes (4 °C, 8 °C and 4 °C with a reconditioning to reach 15 °C at four months of storage) (N = 2 years).

Total RS (glucose + fructose) and sucrose were analyzed on freeze-dried samples. Five biological replicates (one biological replicate = three tubers) were sampled for each variety, temperature, and observation period over two years of testing. Tubers of each biological sample were peeled, cut in pieces and frozen in liquid nitrogen. Samples were stored at – 80 °C until lyophilized, after which they were kept at ambient temperature until the analysis. RS content was analyzed in tubers of the six tested varieties after four months of storage at 4 or 8 °C and on two varieties stored at 4 °C and reconditioned from 4 to 15 °C to reach 15 °C at four months of storage. The sucrose content was measured only in tubers of two varieties (Verdi and Markies) stored at 4 °C over two months to verify the effect of the variety on sucrose content in tubers stored at low temperature over two seasons of storage.

Sucrose, glucose and fructose were measured using a K-SUFRG kit (Megazyme, Bray, Ireland). Sucrose, D-Fructose and D-Glucose were quantified according to the manufacturer's protocol (Megazyme, K-SUFRG 04/17, Bray, Ireland) with some modifications. Lyophilized samples were crushed in powder and 200 mg of each powder sample were suspended in 1 mL distilled water, vortexed and centrifuged at 10,000 g for 10 min at 4° C. The supernatant of each sample was collected, and sucrose, glucose and fructose content were measured using the microplates protocol of the Megazyme K-SURFG assay procedure 04/17. The 96 wells F-Bottom Microplates (Greiner bio321 one, Frickenhauser, Germany) were used. The Microplate path length was adjusted to 1 cm by dividing the absorbance by 0.625 (= function of the diameter of the well and the total reaction volume). Contents of different sugars (glucose, fructose and sucrose) were calculated as described in the K-SURFG assay procedure 04/17 (Megazyme, Bray, Ireland).

To analyze RS content, a two way linear mixed model was built with the fixed effects: temperature and variety using the "lme 4" R package version 1.1–23 (Bates et al., 2015). The fixed effects were the variety and the temperature. To analyze the sucrose content, a one-way linear model with the variety as fixed effect was used. In both models, the year was considered as the random factor (N = 2) and the effect of the year was removed from the model when the variance of the year was not significant. The "RS" variable was transformed with "log (x + 1)" to ensure the homogeneity of the variance and normality of the response variable. Analysis of RS results were performed separately for the periods: after four months of storage (end of March) in six varieties and two storage temperatures (4 and 8 °C); and after four months of storage in two varieties and three temperature regimes (4 °C, 8 °C and 4 °C with a reconditioning to reach 15 °C at four months of storage) (N = 2 years).

To observe the effect of the fixed effects and interactions for both reducing sugars and sucrose content, we performed significance tests (with a confidence interval of 95 %) using the chi-square test or the F test when the effect of the random factor was not significant, from the "car" R package version 3.0–7 (Fox & Weisberg, 2019).

Tukey's post-hoc (emmeans method) test was performed to decompose significant fixed effects (Lenth, 2020). For the sake of conciseness, in case of significant or low interaction between the factors variety and temperature, the Tukey's test was performed directly on the interaction.

2.2.4. Biochemical measurements: Asparagine content

Asparagine measurements were performed on tubers stored in the "Changins" site. 200 mg of each freeze-dried powder sample was suspended in 1.6 mL of 1 M perchloric acid, mixed and centrifuged at 10,000 g for 10 min at 4° C. 1 mL of supernatant of each sample was collected and transferred into a 2 mL tube. To adjust the pH to 8, 2 M KOH was used and the volume of each tube was made up to 1.5 mL with distilled water. The Microplate procedure was used for asparagine quantification and calculations were performed following the protocol described in the assay procedure K-ASNAM 376 07/17 (the path length was adjusted to 1 cm).

2.2.5. Molecular characterization: VInv transcript levels

Transcript levels of *VInv* measurements were performed on tubers stored in the "Changins" site.

The freeze-dried tuber samples were used for RNA extraction according to the protocol of Kumar, Iyer, and Knowles (2007) with slight modifications. Around 100 mg of freeze-dried tubers were sampled, and the volumes of reagents (NaCl Sodium dodecyl sulfate (SDS), Na_2SO_3, Tris-borate-EDTA (TBE), and 2-sulfanylethanol) were scaled down 5-fold.

RNA quality was verified by agarose gel electrophoresis using a 1 % agarose gel, followed by DNAse treatment using DNAse I (Bioke, Leiden, Netherlands) according to the manufacturer's protocol. To guarantee the total removal of genomic DNA, aliquots of DNAse-treated RNA were again checked on a 1 % agarose gel by electrophoresis. cDNA was synthesized from 500 ng of each DNA-free RNA sample using the GoTaq Reverse Transcription, Oligo dT kit (Promega, Wisconsin, USA) following the manufacturer's instructions. The cDNA was diluted in distilled water 5-fold and 2 μL of each diluted sample was used for a single qRT-PCR reaction with 1x GoTaq qRT-PCR master mix (Promega, Wisconsin, USA) and 10 nM primers for RT-qPCR (StVInv-F, StVInv-R, rRNA-F and rRNA-R).The Primer sequences used are the following: 1) StVinv (forward primer sequence (5′-3′) = GGGTATGTGGGAGTGTGTGG; reverse primer sequence (5′-3′) = ATTCCACAATCCAATTCCGGGT; size = 201 bp) and 2) 18S rRNA (forward primer sequence (5′-3′) = GGGCATTCGTATTTCATAGTCAGAG; reverse primer sequence (5′-3′) = CGGTTCTTGATTAATGAAAACATCCT; size = 101 bp).

Three technical replicates were performed for each sample, and the thermal cycle was performed on a CFX96 Real-Time System (Bio-Rad,

Hercules, USA) using the following program: 95° C for 3 mins initial denaturation, followed by 40 cycles of 95° C for 10 s (denaturation), 60° C for 30 s (annealing) and 72° C for 30 s (elongation), followed by a plate read. Melting curves were generated at temperatures between 65° C and 95° C with 0.5° C increments. The comparative cycle threshold method was employed to calculate the relative gene expression and the expression levels of candidate genes were normalized to the mean delta cycle threshold (dCT) of the housekeeping gene (rRNA). rRNA has been shown to be the best housekeeping gene in conditions of cold treatment of potato tubers (Shumbe, Visse, Soares, Smit, Dupuis, & Vanderschuren, 2020).

Transcript levels of *VInv* were assayed for tubers of the 2017–2018 testing season. Five biological replicates for each variety, temperature and observation period were analyzed. Each biological replicate was composed of a pool of three tubers. We used a two-way Anova to analyze *VInv* transcript data, with fixed effects being temperature and variety. We transformed this variable with "log (x + 1)" to ensure the homogeneity of the variance. Significance tests were performed using F test provided by the "car" R package version 3.0–7 (Fox & Weisberg, 2019). Tukey's post-hoc (emmeans method) test was performed to decompose significant fixed effects (Lenth, 2020). For the sake of conciseness, in case of significant or low interaction between factors variety and temperature, the Tukey's test was performed directly on the interaction.

2.2.6. Statistical analysis

The R software version 3.6.3 (R Core Team, 2019) was used for the statistical analysis. Several R packages were used: "ggplot2", "plyr", "Rmisc", "lattice," and "cowplot" packages for graphics and data summary (Hope, 2013; Sarkar, 2008; Wickham, 2011, 2016; Wilke, 2019) and packages for the statistical analysis are described above. For the different tests, the significance level has been fixed at 5 %.

3. Results

3.1. Low temperature storage delays sprout development

After four and half months of storage (mid-April), sprouting of potatoes was variety-dependent and varied according to the temperature of storage (p-value < 0.001). An interaction was observed between the temperature and variety factors (p-value < 0.001).

Sprout weight was systematically lower in tubers stored at 4 °C compared to the sprout weight of tubers stored at 8 °C, this result was observed for all the tested varieties (p-value < 0.001). Five tubers of LC, Verdi, KB, Pirol, Agria and Markies varieties displayed an average sprout weight of 2.62, 0.17, 1.00, 1.44, 0.00 and 0.21 g, respectively, while five tubers of the same varieties stored at 8 °C had an average sprout weight of 11.42, 8.69, 27.87, 17.71, 3.27 and 8.13 g, respectively (Fig. 1.A), (Fig. 1.B).

The variety effect on sprout weight for tubers stored at 4 °C was different from the variety effect for tubers stored at 8 °C. For tubers stored at 4 °C, the average sprout weight was relatively low for all varieties but the LC variety had a higher sprout weight compared to Verdi, Agria, Markies (p-value < 0.001) and KB varieties (p-value = 0.026). No significant difference in sprout weight was observed between LC and Pirol varieties (p-value = 0.379). In tubers stored at 8 °C, KB was the most sprouted variety with an average sprout weight significantly higher than the sprout weight of other varieties (p-values < 0.001), except for the Pirol variety (p-value = 0.142), probably because the Pirol variety displayed a high sprout weight itself (Fig. 1. B).

3.2. Storage temperature and variety modulate crisp color and acrylamide content

3.2.1. Crisp color quality

After two (end of January) and four months of storage (end of March), the color of crisps was impacted by temperature and variety factors. A significant interaction between temperature and variety effects was detected after two months of storage and disappeared after four months of storage with or without reconditioning (Table 1).Tubers from the six tested varieties displayed a good crisp color quality after two months of storage at 8 °C with an average of at least seven crisps with an acceptable color among 10 crisps evaluated, and no difference in crisp color quality was observed between varieties (lowest p-value = 0.589) (Fig. 2.A). In contrast, the crisp color quality from tubers stored for two months at 4 °C varied according to the variety. Lower numbers of crisps with an acceptable color were obtained with Agria and Markies varieties compared to the numbers of crisps with an acceptable color obtained from the varieties LC, Verdi and KB, stored for two months at 4 °C (highest p-value = 0.012) (Fig. 2.A). Agria and Markies varieties displayed averages of 0.67 and 1.67 crisps, respectively, with an acceptable color among the ten crisps tested; while varieties LC, Verdi and KB displayed averages of 6.67, 7.33 and 7.00 crisps, respectively, with an acceptable color among the ten crisps tested (Fig. 2.A).

The crisp color quality of the Pirol variety was not significantly different to the crisp color quality of varieties LC, KB, Agria, Markies (lowest p-value = 0.106) and Verdi (p-value = 0.055) stored for two

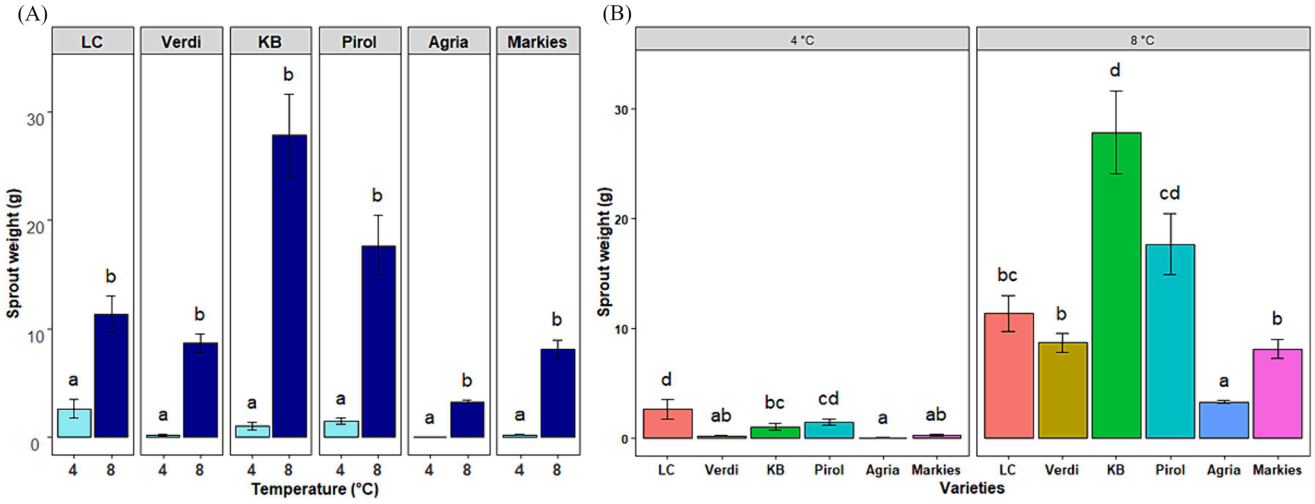

Fig. 1. Effect of the temperature of storage on the sprout weight for each variety (A) and effect of the variety on the sprout weight for the different temperatures of storage (B) after 4.5 months of storage at 4 or 8 °C (80 % RH); over 4 biological replicates (N = 4), mean ± standard error. For a given observation, groups sharing the same letter are not significantly different (Tukey's test, confidence level of 95 %); (LC = Lady Claire; KB = Kiebitz). Color should be used for this figure in print.

Table 1
P-values from significance tests (Pr[>chi-sq] or P[F test]) for the measured parameters in response to the different factors and their interactions after two and four months of storage at 4 and 8 °C on six varieties and after a storage at 4 °C with reconditioning from 4 to 15 °C to reach 15 °C at four months of storage on two varieties (* = statistically significant; (*) = low significance; NA = not analyzed, statistical analyses are described in section 2.2 for each measurement).

Factors	Period of observation in months (m.) (+number of varieties tested)	Crisp color	Reducing sugars content	Sucrose content	*Vacuolar invertase* gene expression	Asparagine content
Temperature	2 m. (6 varieties)	<0.001*	NA	NA	<0.001*	0.177
	4 m. (6 varieties)	<0.001*	<0.001*	NA	0.003*	NA
	4 m. + reconditioning (2 varieties)	<0.001*	0.001*	NA	<0.001*	NA
Variety	2 m. (6 varieties)	<0.001*	NA	<0.001* (2 varieties)	0.005*	0.023*
	4 m. (6 varieties)	<0.001*	<0.001*	NA	0.107	NA
	4 m. + reconditioning (2 varieties)	<0.001*	<0.001*	NA	0.011*	NA
Temperature X variety	2 m. (6 varieties)	0.027*	NA	NA	0.083(*)	0.040*
	4 m. (6 varieties)	0.535	0.005*	NA	0.200	NA
	4 m. + reconditioning (2 varieties)	0.326	0.009*	NA	0.052(*)	NA

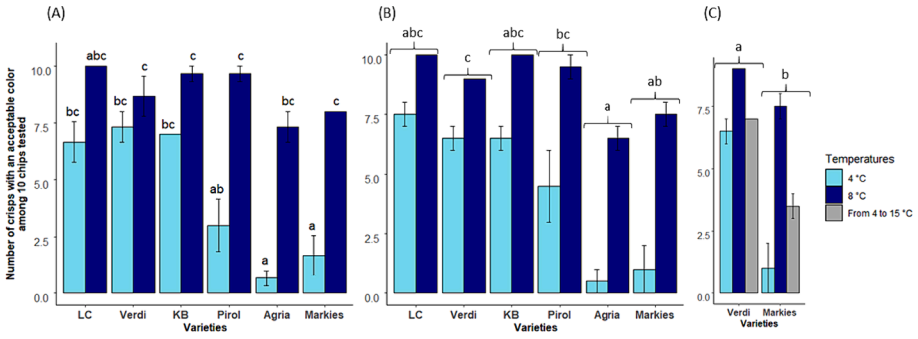

Fig. 2. Average number of crisps with an acceptable color among the 10 crisps tested for the six varieties stored at 4 or 8 °C during two months (A) (N = 3 years, Tukey's test, groups sharing the same letter are not significantly different, confidence level of 95 %), four months (B) (N = 2 years) and for two varieties stored at 4, 8 °C and reconditioned from 4 to 15 °C to reach 15 °C at four months of storage (C) (N = 2 years), ± standard error, LC = Lady Claire and KB = Kiebitz. Color should be used for this figure in print.

months at 4 °C. It should be noted that the crisp color quality from tubers of the Verdi variety seemed higher than that from tubers of the Pirol variety stored at 4 °C, and that even though the effect was not significant, the p-value was low (p-value = 0.055) (Fig. 2.A). The Verdi variety displayed an average of 7.33 crisps with an acceptable color among the ten crisps tested, while Pirol variety displayed an average of 3 crisps with an acceptable color among the ten crisps tested (Fig. 2.A).

Crisps obtained from tubers of LC, Verdi and KB varieties stored at 4 °C had a similar quality compared to crisps obtained from tubers of the same varieties stored at 8 °C for two months (lowest p-value = 0.471). In contrast, the varieties Pirol, Agria and Markies displayed a lower number of crisps with an acceptable color after two months of storage at 4 °C compared to the crisp color from tubers of the same varieties stored for two months at 8 °C (p-values = 0.006, 0.001 and 0.001) (Fig. 2.A). Varieties Pirol, Agria and Markies displayed an average of 3, 0.67 and 1.67 crisps respectively, with an acceptable color among 10 crisps tested after two months of storage at 4 °C, and of 9.67, 7.33 and 8 crisps, respectively, with an acceptable color among 10 crisps tested after two months of storage at 8 °C (Fig. 2.A).

After four months of storage, trends were similar to the observation at two months of storage; however, the interaction between temperature and variety factors could not be shown statistically (Table 1). The number of crisps with an acceptable color was almost two-fold higher in crisps from tubers stored at 8 °C compared to crisps from tubers stored at 4 °C for four months (Table 1). On average, tubers of the six varieties displayed 8.75 and 4.42 crisps with an acceptable color among the 10 crisps tested after four months of storage at 8 °C and 4 °C, respectively. The crisp color quality varied according to the variety after four months of storage (Table 1).

The number of crisps with an acceptable color quality obtained from tubers of the Verdi variety after four months of storage was significantly higher than the number of crisps with an acceptable color quality obtained from tubers of the Agria and Markies varieties (p-values = 0.004 and 0.024). Verdi displayed an average of 7.75 crisps with an acceptable color among the 10 crisps tested, while Agria and Markies displayed an average of 3.5 and 4.25 crisps, respectively, with an acceptable color among the 10 crisps tested (Fig. 2.B).

The Pirol variety had a significantly higher number of crisps with an acceptable color (average of 7.00 crisps with an acceptable color among the 10 crisps tested) compared to the variety Agria (p-value = 0.018) (Fig. 2.B).

The varieties LC and KB also had high numbers of crisps with acceptable color (average of 8.75 and 8.25 crisps with an acceptable color among 10 crisps tested, respectively). However, the Tukey's test did not discriminate the crisp color quality of these varieties from the other varieties (lowest p-value = 1) (Fig. 2.B).

The crisp color quality of the varieties Markies and Verdi after four months of storage at three temperature regimes: 8 °C, 4 °C and at 4 °C with a reconditioning from 4 to 15 °C (Fig. 2.C) was affected by the temperature and variety factors (Table 1). There was no interaction between those two factors (Table 1). On average, the crisp color quality was significantly higher for tubers stored at 8 °C (average 8.28 crisps with an acceptable color among the 10 crisps tested) than at 4 °C (average of 3.75 crisps with an acceptable color among the 10 crisps tested) (p-value = 0).

The crisp color quality from tubers reconditioned from 4 to 15 °C (average of 5.25 crisps with an acceptable color among the 10 crisps tested) seemed higher than the crisp color quality of crisp from tubers stored at 4 °C (p-value = 0.234), however Tukey's test did not discriminate the color quality of crisps stored at these two temperature regimes (p-value = 0.234) (Fig. 2.C).

On average, the variety Verdi displayed a higher crisp color quality

compared to the crisp color quality of the Markies variety (p-value = 0). Verdi had an average of 7.5 crisps with an acceptable color among the 10 crisps tested and Markies had an average of 4 crisps with an acceptable color among the 10 crisps tested (Fig. 2.C).

3.2.2. Acrylamide content in crisps

Trends indicate that for the six tested varieties, the acrylamide content was lower in crisps from tubers stored at 8 °C (average of 627.25 μg kg^{-1}) than in crisps from tubers stored at 4 °C (average of 3611.08 μg kg^{-1}). This difference was less pronounced in crisps from the varieties LC, Verdi and KB in which the acrylamide content remained relatively low and similar after four months of storage at 4 °C (average of 1339, 334.50 and 1234 μg kg^{-1}, respectively), compared to the acrylamide content in crisps from tubers of the same varieties stored at 8 °C (average of 560.50, 220, 354.50 μg kg^{-1}, respectively). In contrast, in tubers from Pirol, Agria and Markies varieties, the acrylamide content was higher in crisps from tubers stored at 4 °C (average of 2486, 7924 and 8349 μg kg^{-1}, respectively) compared to crisps from the tubers stored at 8 °C (average of 364, 1400.50 and 864 μg kg^{-1}).

Trends also suggest that the reconditioning from 4 to 15 °C decreases the acrylamide content of 75 % in crisps from tubers of Markies variety (average of 2100 μg kg^{-1}) compared to the acrylamide content in crisps from tubers of the same variety stored for four months at 4 °C without reconditioning (average of 8349 μg kg^{-1}). However, the reconditioning did not allow tubers to reach an acrylamide content as low as in crisps from tubers of the variety Markies stored at 8 °C (average of 864 μg kg^{-1}). The reconditioning had a lower impact on the acrylamide content in crisps from tubers of the Verdi variety (average of 178.67 μg kg^{-1}) because the acrylamide content in this variety was already low even after storage at 4 °C (Fig. 3).

3.3. Biochemical measurements: Sugar content in tubers is variety and temperature dependent

3.3.1. Glucose analysis in tubers

The glucose content in tubers from varieties Agria, Pirol and Markies after two months of storage at 4 °C was high and unacceptable in most tubers (70 %, 100 % and 100 % of data indicated a glucose content > 75 mg dL^{-1}). Conversely, tubers of the same varieties stored at 8 °C had a low and acceptable glucose content (≤75 mg dL^{-1}) (0 % of data indicated a glucose content > 75 mg dL^{-1}). For these three varieties, the confidence intervals at 4 or 8 °C did not overlap, meaning that there is a clear difference in glucose content between the two temperatures of storage

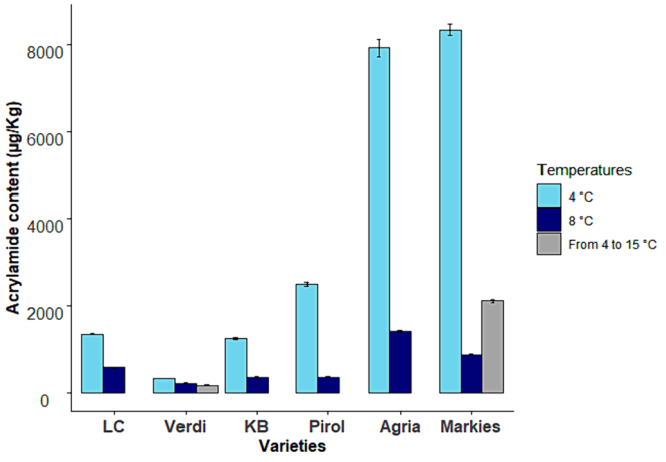

Fig. 3. Average acrylamide content in crisps from the season 2017–2018 (N = 2 to 3 analytical replicates), in six varieties stored at 4 or 8 °C for four months and in crisps from Markies and Verdi varieties stored at 4 °C and reconditioned from 4 to 15 °C to reach 15 °C at four months of storage, ± standard error, LC = Lady Claire and KB = Kiebitz. Color should be used for this figure in print.

(Fig. 4.A).

Tubers of LC and Verdi varieties displayed a low and acceptable glucose content at both 4 and 8 °C (0 % of data indicated a glucose content > 75 mg dL^{-1}). In tubers of the KB variety, the glucose content was low and no clear difference in glucose content was observed in tubers of this variety stored at 4 °C (20 % of data indicated a glucose content > 75 mg dL^{-1}) or at 8 °C (0 % of data indicated a glucose content > 75 mg dL^{-1}), the confidence intervals at 4 and 8 °C overlap (Fig. 4.A).

After four months of storage, tubers of the Pirol, Agria and Markies varieties stored at 4 °C had an unacceptable glucose content (70 %,100 % and 100 % of data, respectively, indicating a glucose content > 75 mg dL^{-1}), while tubers of the same varieties stored at 8 °C had an acceptable glucose content (≤75 mg dL^{-1}) (0 %, 10 % and 10 % of data indicated a glucose content > 75 mg dL^{-1} respectively). For these three varieties, the confidence intervals estimated by the Agresti-Coull function at 4 or 8 °C did not overlap. Based on this method (Agresti & Coull, 1998), this suggests that there is a clear difference in glucose content between the two temperatures of storage (Fig. 4.B). In contrast, the varieties LC, Verdi and KB stored for four months have an acceptable glucose content at both 4 and 8 °C (0 % of data indicated a glucose content > 75 mg dL^{-1} for the three varieties).

After four months of storage the glucose content was always low in tubers of the Verdi variety stored at 4 °C, 8 °C and 4 °C with a reconditioning at 15 °C (0 % of data indicated a glucose content > 75 mg dL^{-1} for the three varieties) (Fig. 4.C). Conversely, the glucose content in tubers of the Markies variety was unacceptable after a storage of four months at 4 °C (100 % of data indicated a glucose content > 75 mg dL^{-1}), while the glucose content was lower in tubers of this variety stored at 8 °C or at 4 °C with a reconditioning at 15 °C (only 10 % of data indicated a glucose content > 75 mg dL^{-1}) (Fig. 4.C).

3.3.2. Reducing sugars in tubers

RS content (glucose + fructose) was analyzed in tubers of the six varieties after four months of storage at 4 and 8 °C and in tubers of the varieties Markies and Verdi after a reconditioning from 4 to 15 °C. After four months of storage with or without reconditioning, RS content was significantly affected by the temperature of storage and varied according to the variety. There was an interaction between temperature and variety factors (Table 1).

In tubers stored at 8 °C, the RS content was lower in tubers of the LC variety compared to the RS contents in tubers of the Verdi, Agria and Markies varieties (p-value = 0.013, p-value < 0.001 and p-value < 0.001) and was not different to the RS contents in tubers of the KB and Pirol varieties (p-value = 0.237 and p-value = 0.116).

The RS content in tubers of the Agria variety stored at 8 °C was higher than that in tubers of the LC, Verdi, Pirol and Markies varieties (p-value < 0.001; p-value < 0.001; p-value < 0.001 and p-value = 0.004) and was not different from that in tubers of the KB variety (p-value = 0.253) (Fig. 5.A).

RS contents in tubers of LC, Verdi and KB varieties were low and equal in tubers stored at both 4 °C (average of 71.97; 103.73 and 274.3 mg/100 g DW) and 8 °C (average of 37.48; 95.63 and 138.13 mg/100 g DW) (lowest p-value = 0.409).

Conversely, RS contents in tubers of Pirol and Markies varieties stored at 4 °C (average of 328.25 and 266.92 mg/100 g DW) were significantly higher compared to the RS contents in tubers of the same varieties stored at 8 °C (average of 82.98 and 150.05 mg/100 g DW) (p-value < 0.001 and p-value = 0.042). RS content in tubers of the Agria variety stored at 4 °C seemed higher (average of 633.92 mg/100 g DW) compared to RS content in tubers of the same variety stored at 8 °C (average of 322.76 mg/100 g DW), however the Tukey's test did not discriminate differences in RS content between storage at 4 or 8 °C for this variety (p-value = 0.298) (Fig. 5.A).

After four months of storage at 4 °C, the RS contents in tubers of the LC and Verdi varieties were equivalent and low (p-value = 0.782) and were significantly lower than the RS content in tubers of the KB, Pirol,

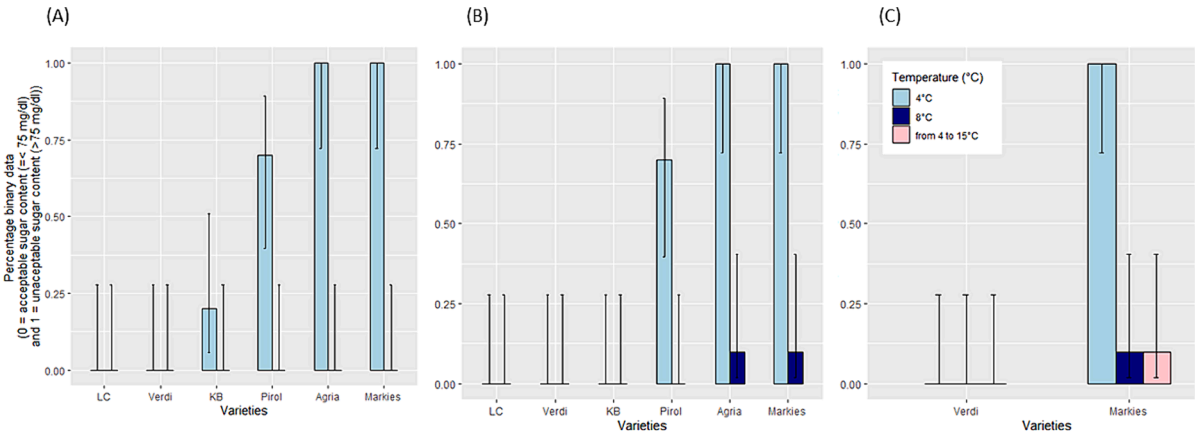

Fig. 4. Glucose content represented by the percentage of binary data with an unacceptable glucose content (>75 mg dL^{-1}) (N = 10, 5 biological replicates x 2 years), for the six tested varieties stored at 4 and 8 °C for two months (A), four months (B) and for the two tested stored at 4, 8 °C and reconditioned from 4 to 15 °C at four months of storage (C) + lower and upper confidence intervals estimated by the Agresti-Coull function (Agresti & Coull, 1998). Color should be used for this figure in print.

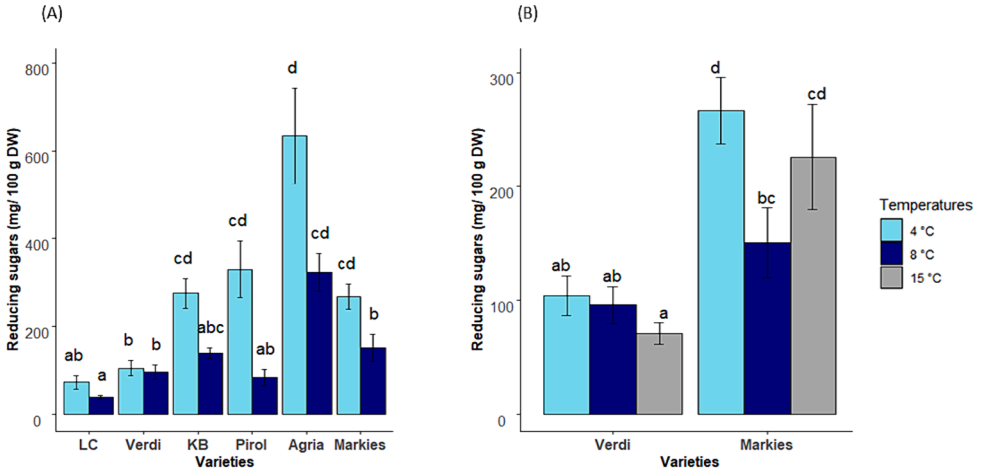

Fig. 5. Average reducing sugars content in six potato varieties stored at 4 or 8 °C for four months (A) (N = 2 years, Tukey's test, groups sharing the same letter are not significantly different, confidence level of 95 %), and in two varieties stored at 4, 8 °C and reconditioned from 4 to 15 °C during four months (B) (N = 2 years), ± standard error, LC = Lady Claire and KB = Kiebitz. Color should be used for this figure in print.

Agria and Markies varieties stored at the same temperature (p-value < 0.001) (Fig. 5.A).

Fig. 5.B shows that the RS content was low and equal in tubers of the Verdi variety stored at 4 °C, 8 °C and 4 °C with a reconditioning from 4 to 15 °C over four months (lowest p-value = 0.533). RS content was higher in tubers of the Markies variety stored at 4 °C compared to the RS content in tubers of the same variety stored at 8 °C and compared to the RS content in tubers of the Verdi variety stored at 4 °C or 8 °C (p-value = 0.001). RS content was not significantly lower in tubers of Markies variety reconditioned from 4 to 15 °C compared to RS in tubers of Markies stored at 4 °C (p-value = 0.408).

3.3.3. Sucrose content in tubers

The sucrose content in tubers of the Markies and Verdi varieties after two months of storage at 4 °C (until end of January) was measured to evaluate the sugar "stocks" in these varieties stored at low temperature. The sucrose content was significantly lower in tubers of the Markies variety (average of 460.7 mg/100 g DW) than in tubers of the Verdi variety (average of 1385.92 mg/100 g DW) (Table 1).

3.4. Genetic and enzymatic measurements

3.4.1. Vacuolar invertase gene expression is temperature and variety dependent

The transcript levels of *VInv* were evaluated by RT-qPCR after two months (until end of January) and four months (until end of March) of storage at 8 °C and 4 °C, and after a storage at 4 °C with a reconditioning to reach 15 °C at four months of storage. After two months of storage (during the 2017–2018 season of storage), transcript levels of *VInv* varied according to the temperature of storage, the variety, and there is a marginal interaction between these two factors (p = 0.083) (Table 1). Transcript levels of *VInv* were relatively low and similar in tubers stored at both 4 °C and 8 °C for varieties LC and Verdi (p-values = 1 and 0.999 respectively). Transcript levels of *VInv* for the KB variety were similar in tubers stored at both 4 and 8 °C (p-value = 0.707). However, transcript levels of *VInv* in tubers of the KB variety seemed high after two months of storage at both 4 °C and 8 °C, compared to other varieties. Indeed, transcript levels of *VInv* in tubers of the KB variety stored at 8 °C for up to two months was significantly higher than the transcript levels in tubers of the Agria variety stored at the same temperature (p-value = 0.042). After a storage at 4 °C, the transcript levels of *VInv* in tubers of the KB variety were significantly higher than in tubers of the LC variety stored

at 4 °C (p-value = 0.047). Finally, the Tukey's test did not discriminate the transcript levels of *VInv* in tubers of the KB variety from the transcript levels of *VInv* in tubers of the other varieties for storage at 4 °C (lowest p-value = 0.188) or 8 °C (lowest p-value = 0.334) (Fig. 6.A).

The transcript levels of *VInv* in tubers of the Agria variety stored at 4 °C were significantly higher compared to the *VInv* gene expression in tubers of the same variety stored at 8 °C (p-value = 0.01). The transcript levels of *VInv* in tubers of the Markies variety seemed higher in tubers stored at 4 °C compared to the transcript levels of *VInv* in tubers of the same variety stored at 8 °C. However, the Tukey's test did not discriminate the transcript levels of *VInv* in tubers of the Markies variety stored at 4 °C from the transcript levels of *VInv* in tubers of this variety stored at 8 °C (p = 0.534). No significant differences in transcript levels of *VInv* were observed in tubers of the Pirol variety stored at 4 °C compared to the transcript levels of *VInv* in tubers of the same variety stored at 8 °C (p-value = 0.772) (Fig. 6.A).

After four months of storage, the transcript levels of *VInv* varied according to the temperature of storage. There was no impact of the variety and no interaction between variety and temperature factors (Table 1). The transcript levels of *VInv* were significantly higher in tubers stored at 4 °C (average relative mRNA of *VInv* of 6.38) compared to 8 °C (average relative mRNA of *VInv* of 3.96) (Table 1).

The study of the effect of four months of storage at 4 °C, 8 °C and 4 °C with a reconditioning at 15 °C in tubers of Verdi and Markies varieties showed that the transcript levels of *VInv* varied according to the temperature and variety, and that there is a low interaction between the temperature and the variety (Table 1). After four months of storage, the transcript levels of *VInv* were significantly higher in tubers of the Markies variety stored at 4 °C (average relative mRNA of *VInv* of 9.28) compared to the transcript levels of *VInv* in tubers of Markies stored at 8 °C (average relative mRNA of *VInv* of 2.62) or 4 °C with a reconditioning at 15 °C (average relative mRNA of *VInv* of 1.90). Consequently, the reconditioning after a storage of four months at 4 °C allowed a decrease of 80 % in average relative mRNA of *VInv* compared to a storage at 4 °C without reconditioning for the variety Markies. Transcript levels of *VInv* in tubers of Markies variety stored at 4 °C were also higher compared to the transcript levels of *VInv* in tubers of the Verdi variety stored at 4 °C, 8 °C and at 4 °C with a reconditioning at 15 °C (highest p-value = 0.029) (Fig. 6.B).

3.4.2. *Asparagine content in tubers*

After two months of storage (season 2017–2018), the asparagine content was not affected by the storage temperature (Table 1), however it was affected by the variety and this effect was temperature-dependent (Table 1). In tubers stored at 4 °C, the asparagine content was similar among the six tested varieties (lowest p-value = 0.216), while in tubers stored at 8 °C, the asparagine content was significantly higher in tubers of the KB variety (average of 512.64 mg/100 g DW) compared to asparagine content in tubers of the Pirol and Agria varieties (average of 355.35 and 350.30 mg/100 g DW respectively) (p = 0.039 and 0.050 respectively). The asparagine content in tubers of the KB variety stored at 8 °C was equivalent to the asparagine content in tubers of LC, Verdi and Markies varieties stored at the same temperature (average of 475.20, 436.51 and 411.99 mg/100 g DW respectively) (lowest p-value = 0.415) (Fig. 7).

4. Discussion

The recent non-renewal of the use of chlorpropham molecule to control potato sprouting in Europe is prompting the potato value chain to find storage alternatives to control sprouting (European Food Safety Authority EFSA, 2017; European Commission, 2019). To avoid using other anti-sprouting chemicals, often less efficient than CIPC (Visse-Mansiaux et al., 2020), the storage at low temperatures to control sprouting should be considered for certain varieties.

Potato dormancy is mainly influenced by the genotype and the temperature of storage (Daniels-Lake & Prange, 2007; Magdalena & Dariusz, 2018; Sharma, Sucheta, & S., & Yadav, S. K. , 2020; Visse-Mansiaux et al., 2022). This has been confirmed in our study as

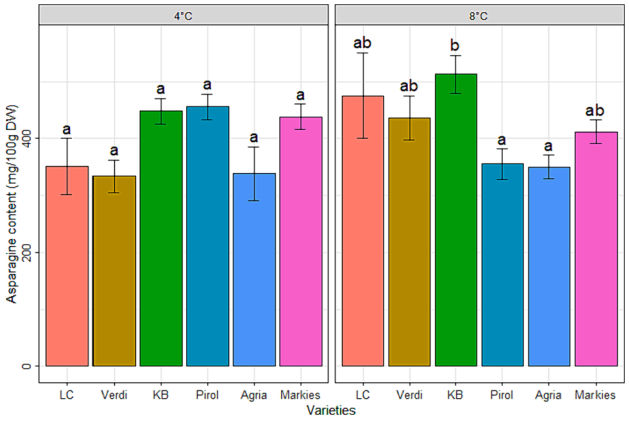

Fig. 7. Effect of variety on the asparagine content in tubers stored at 4 °C, and effect of the variety in tubers stored at 8 °C during two months; mean ± standard error, Tukey's test, groups sharing the same letter are not significantly different (confidence level of 95 %), (N = 5 biological replicates over 1 year).

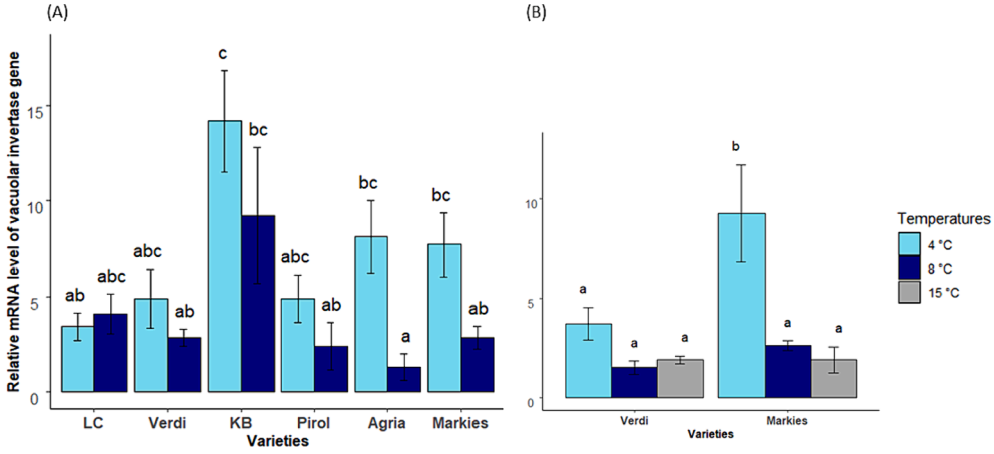

Fig. 6. Relative mRNA level of *vacuolar invertase* gene (*VInv*) in six potato varieties stored at 4 or 8 °C over two months (A), and in two varieties stored at 4, 8 °C and reconditioned from 4 to 15 °C after four months (B), ± standard error, LC = Lady Claire and KB = Kiebitz, (N = 5 biological replicates over one season of storage, Tukey's test, groups sharing the same letter are not significantly different, confidence level of 95 %). Color should be used for this figure in print.

varieties displayed differences in sprout weight after 4.5 months of storage (until mid-April) and the effect of the variety on sprouting was shown to be temperature-dependent. The storage at low temperature (*i.e.* 4 °C) was more efficient in reducing sprouting compared to storage at higher temperature (*i.e.* 8 °C) for the six tested varieties up to 4.5 months of storage. LC, Verdi, KB, Pirol, Agria and Markies varieties displayed 77, 98, 96, 92, 100 and 97 % of sprout weight reduction, respectively. Our results are in line with the literature reporting that low temperatures during storage allow efficient sprout control (Burton & Wilson, 1978; Paul et al., 2016; Sonnewald, 2001; Sowokinos, 2001). For example, Gichohi and Pritchard (1995) reported that sprouting of the Shepody potato variety was delayed by 15 weeks when the temperature was lowered from 8 to 5 °C.

To store potatoes at low temperatures, the variety should be selected with caution due to the susceptibility of certain varieties to CIS, which can lead to dark color and bitter-tasting of French fries and crisps (Amjad et al., 2020; Mottram et al., 2002; Pinhero et al., 2011). CIS may also lead to the production of toxic compounds such as acrylamide in tubers during frying, which may raise concerns for human health (FAO/WHO, 2002; Paul et al., 2016; Wiberley-Bradford & Bethke, 2017; Wiberley-Bradford et al., 2016).

The frying quality was influenced by the storage temperature for certain varieties. The varieties Agria, Pirol and Markies obtained poor results with a higher number of dark crisps after two months of storage (until end of January) at 4 °C compared to 8 °C. Conversely, the LC, Verdi and KB varieties had better crisp quality results with good crisp color quality after two months of storage at both 4 and 8 °C. After four months of storage, trends were similar, although the effect of variety by temperature interaction on crisps color quality could not be statistically proven. This lack of significance at four months of storage is probably because of the use of binomial data, which are less powerful than conventional data. Consequently, after four months of storage, only the global negative effect of temperature and the effect of the variety on crisps color quality was demonstrated.

Our results are in line with the literature as the variety by temperature interaction effect on fry color has been previously reported. Groza et al. (2006) reported that the White Pearl variety produced light crisps after a storage at 3.3 °C, while four other varieties tested in their study produced darker crisps after storage at the same temperature. They also reported that a 1-month reconditioning at 12.8 °C following storage at 3.3 °C for 6 months returned the crisps color quality of the White Pearl variety to the same level than after storage at warmer temperatures. Our results obtained with the variety Markies also suggest that a reconditioning from 4 to 15 °C increases the crisp color quality. However, this effect was not statistically significant.

The darkening of crisps was associated with an increase in acrylamide content. After four months of storage, trends indicated that the acrylamide content was higher in crisps from tubers stored at 4 °C compared to 8 °C. In addition, differences in acrylamide content between a storage at 4 °C and a storage at 8 °C were much higher in crisps obtained from tubers of Pirol, Agria and Markies varieties, than in crisps obtained from tubers of LC, Verdi and KB varieties. Higher acrylamide contents in crisps of 583, 466, 866, 139, 52 and 248 % have been observed from tubers stored at 4 °C compared to acrylamide in crisps from tubers stored at 8 °C for four months for varieties Pirol, Agria, Markies, LC, Verdi and KB, respectively. The reconditioning from 4 to 15 °C allowed a decrease in acrylamide content in tubers of the Markies variety of 75 % compared to the acrylamide content in tubers of this variety stored at 4 °C for four months. However, the acrylamide content in reconditioned tubers is 143 % higher than the acrylamide content in tubers of this variety stored at 8 °C.

The poor crisp color quality displayed by varieties Pirol, Agria and Markies after two months of storage, with similar trends after four months of storage, was correlated with the trends that indicated a high acrylamide content in these varieties after four months of storage at 4 °C. Our results are in line with those of Matsuura-Endo et al. (2006). They studied the effect of storage temperature on four potato varieties and reported an increase of dark brown crisp color, acrylamide and RS for all varieties, when stored at temperatures lower than 8 °C.

Reaction between amino acid (mainly asparagine) and RS through the Maillard reaction during frying of potatoes is responsible for the increase in acrylamide content and for the darkening after frying (Mottram et al., 2002).

In our study, the asparagine content in tubers did not explain the differences observed in crisp color and acrylamide content between tubers stored at 4 °C instead of 8 °C, as the asparagine content was not affected by the temperature of storage. However, the asparagine content was affected by the genotype in a temperature-dependent manner. After two months of storage at 4 °C, the asparagine content in tubers was not different between varieties, while at 8 °C, the asparagine content was higher in the variety KB compared to the varieties Pirol and Agria. Our results are in line with a recent study reporting the impact of cold temperature (5 °C) storage on potato tubers (variety "Michuñe negra") grown during two different seasons (winter or summer) (García-Ríos et al., 2023). Both season and duration of storage at cold temperature had a significant impact on the RS levels but not on asparagine levels, indicating that both factors have a direct effect of the acrylamide formation potential through RS accumulation.

Our findings suggest that darkening of crisps is correlated with high acrylamide, high glucose and/or high total RS sugar content in tubers stored at low temperature. Our results are in line with Liyanage, Yevtushenko, Konschuh, Bizimungu, and Lu (2021) showing that the correlation between RS and acrylamide formation is stronger than the correlation between asparagine and acrylamide formation.

In addition to displaying good crisp color quality after storage at both 4 and 8 °C after two months, with similar trends after four months, LC, Verdi and KB varieties had low glucose content after two and four months of storage at 4 °C ($\leq$75 md dL^{-1} for 100 % of data). For these varieties, the glucose content, as well as the total RS content were not higher in tubers stored at 4 °C than in tubers stored at 8 °C. Furthermore, LC and Verdi displayed low RS content after four months of storage at 4 °C. For the KB variety, even if the RS content was not higher after storage at 4 °C than at 8 °C, RS content in this variety stored at 4 °C was higher than in LC and Verdi varieties stored at 4 °C.

Based on these results, we concluded that LC, Verdi and KB varieties can be identified as not susceptible to CIS, or "CIS-resistant varieties". Verdi has been reported in the literature to be a CIS-resistant variety (Böhm et al., 2006; Fischer et al., 2013; Shumbe et al., 2020), as well as LC variety (Fischer et al., 2013). To our knowledge our study is the first scientific study identifying the KB variety as CIS-resistant.

Inversely, the three varieties Pirol, Agria and Markies displayed poor crisp color quality after two months of storage at 4 °C compared to 8 °C, with similar trends after four months. In addition, in tubers of these three varieties, the storage at low temperature led to an unacceptable glucose content (*i.e.* $>$ 75 mg dL^{-1}) in comparison with glucose content in tubers of the same varieties stored at 8 °C (*i.e.* $\leq$ 75 md dL^{-1}) for up to four months of storage. Levels higher than 75 mg dL^{-1} of glucose in potatoes are not acceptable by the industry for processing potatoes (Olsen et al., 2011). Based on these results, we concluded that Pirol, Agria and Markies are susceptible to CIS and can be considered as "CIS-susceptible varieties". Furthermore, higher total RS content was also observed in tubers of Pirol and Markies CIS-susceptible varieties stored at 4 °C compared to 8 °C but not in tubers of the CIS-susceptible Agria variety. Our results are in line with the literature stating that crisp color quality is inversely correlated with the glucose content in tubers (Coleman, Tai, Clayton, Howie, & Pereira, 1993). Consequently, a solution could be to store CIS-resistant varieties at low temperatures and CIS-susceptible varieties at 8 °C with anti-sprouting treatments to maintain potato quality. Mint essential oil could be used to decrease sprouting as it has been shown that this product does not affect acrylamide formation or RS content in potatoes and thus is suitable for potatoes used by the processing industry (Bruno et al., 2023).

Another solution would be to use the reconditioning after storage at low temperature as a strategy to decrease the RS content in tubers stored at low temperature and to improve the quality of fried potatoes. De Wilde et al. (2005) showed that a reconditioning from 4 to 15 °C was efficient to decrease the RS content in potato tubers after storage at low temperature, and Schippers (1975) reported a positive effect of reconditioning after storage at 5 °C on the crisp quality. In our study, the positive effect of reconditioning on the glucose content was also observed with the variety Markies. This CIS-susceptible variety could be stored at 4 °C with a limited sweetening by applying a reconditioning in temperature from 4 to reach 15 °C after four months of storage. The glucose content in tubers of the Markies variety was unacceptable after a storage of four months at 4 °C (*i.e.* > 75 mg dL^{-1}), while the glucose content was lower and similar in tubers of this variety stored at 8 °C or at 4 °C with a reconditioning at 15 °C. At the opposite of the glucose content results, the reconditioning of tubers from the CIS-susceptible Markies variety did not result in a significant decrease in RS content.

In general, our results underline a variety-dependent effect of the storage temperature on glucose content, acrylamide content and crisp color quality. Our results are in line with De Wilde et al. (2005) who reported that Saturna was the least susceptible variety to acrylamide formation during frying compared with Bintje and Ramos. The authors further showed that storage at low temperature (*i.e.* 4 °C) increases RS content and acrylamide formation compared to storage at 8 °C. In our study, the impact of low temperature as well as the effect of the reconditioning on the glucose content was clearer than the effects on the total RS content. These results are in line with Pritchard and Adam (1994) who showed that the fry color of the Russet Burbank variety correlated with the glucose content and that the relation with the glucose content was stronger than with the fructose or total RS content.

The increase in glucose content observed after two and four months of storage at low temperature in CIS-susceptible varieties probably originates from the cleavage of sucrose in RS by acid invertase (McKenzie et al., 2013). The sucrose content in tubers of the CIS-susceptible Markies variety stored at 4 °C for two months was lower than the sucrose content in tubers of the CIS-resistant Verdi variety stored at the same temperature. The sucrose "stock" in Markies has probably been converted in RS under the effect of low temperature. Conversely, in tubers of the Verdi variety, the sucrose "stock" was maintained high, probably because a low amount of sucrose was converted in RS, as this variety is CIS-resistant. Our results are in line with Sowokinos (2001) who reported that during the CIS phenomenon in potato tubers, several enzymatic reactions occur and the rate of starch conversion to sucrose and of sucrose to RS is accelerated. As certain varieties may accumulate higher levels of sucrose (Gikundi, Buzera, Orina, & Sila, 2023) which may lead to CIS at low temperature, it would be of interest to measure the sucrose "stock" kinetic during storage in different varieties in further studies.

The acid invertase activity level mainly controlled the hydrolysis of sucrose to RS (Pressey & Shaw, 1966; Sowokinos, 2001; Stitt & Sonnewald, 1995) and the *VInv* gene has been reported to play an important role in potato CIS (Bhaskar et al., 2010). After two months of storage, in tubers of LC and Verdi, the transcript levels of *VInv* were low and similar in tubers stored at 4 or 8 °C, consequently the conversion of sucrose to RS was probably mitigated, which could explain the low glucose content in these CIS-resistant varieties. Our results are in line with Shumbe et al. (2020), reporting that transcript levels of *VInv* in the CIS-resistant variety Verdi were lower than in CIS-susceptible varieties. Those results indicate variation between CIS-susceptible varieties. Our results are in line with the literature as it has been reported that CIS may be due to genetic factors such as mutations in the regulator elements of the promoter or in the coding sequence affecting the activity of the Vinv enzyme (Shumbe et al., 2020).

Surprisingly, the transcript levels of *VInv* in tubers of the CIS-resistant KB variety stored for two months at 4 or 8 °C seemed high. Indeed, the *VInv* gene was overexpressed in tubers of the CIS-resistant variety KB stored for two months at 4 or 8 °C, compared to the transcript levels of *VInv* in tubers of the CIS-susceptible variety Agria stored at 8 °C. This result means that even if the transcript levels of *VInv* were high, the invertase enzyme was not produced systematically. One possibility is that invertase inhibitors could block the activities of the enzyme invertase even if the *VInv* gene was overexpressed and Vinv enzyme produced. There have been previous reports about inhibitors of invertase (Greiner, Rausch, Sonnewald, & Herbers, 1999) and further studies would need to be conducted to determine if such inhibiting activities exist in potato and partially explain a reduced Vinv activity despite induction of *Vinv* expression and accumulation of Vinv enzyme.

After two months of storage, the *VInv* gene was overexpressed in tubers of the CIS-susceptible Agria variety after storage at 4 °C compared to 8 °C, and thus the increase in glucose content observed in tubers of this variety stored at low temperature was probably due to a high conversion of sucrose to RS through the invertase protein under control of the *VInv* gene.

No overexpression of the *VInv* gene was observed in tubers of the Pirol CIS-susceptible variety stored at 4 °C for two months compared to the gene expression in tubers of the same variety stored at 8 °C. This implies that other mechanisms may be involved in the increase of glucose content observed after storage at low temperatures with this variety. Indeed, it is reported that invertases are involved in the regulation of the ratio of hexose to sucrose but do not control the total amount of soluble sugars in potatoes stored at low temperature (Zrenner, Schüler, & Sonnewald, 1996).

After four months of storage, higher transcript levels of the *VInv* gene were observed in tubers of the CIS-susceptible Markies variety stored for four months at 4 °C, compared to the transcript levels of *VInv* in tubers of this variety stored at 8 °C. In addition, results revealed that a reconditioning at 15 °C after a storage at 4 °C significantly reduced the transcript levels of *VInv*. The reconditioning resulted in transcript levels of *VInv* after four months of storage in tubers of the Markies variety similar to the level of transcripts of *VInv* in tubers of the same variety stored at 8 °C, or in tubers of the CIS-resistant Verdi variety stored for four months at 4 °C or 8 °C.

In addition to a decrease in the glucose and acrylamide content in tubers, results showed that the reconditioning from 4 to 15 °C after four months of storage also significantly decreased the transcript levels of *VInv* in the CIS-susceptible Markies variety in comparison to storage at 4 °C without reconditioning. Our results are in line with Knowles, Driskill, and Knowles (2009), who reported that a reconditioning at 16 °C allows a RS decrease by accelerating the catabolism of RS and thus restoring processing quality. The gradual increase in temperature from 4 °C to 15 °C leads to an increase in metabolic and biochemical activities in the Markies potato tubers. The raise in temperature during reconditioning increases the respiration rate in potatoes and thus the conversion of sugars. In addition, increasing the temperature can also activate enzymes converting RS into starch (De Wilde et al., 2005; Geigenberger, 2003).

However, it would be necessary to study the reconditioning potential of potato varieties before storage at low temperatures with a reconditioning, as it is reported that the effect of reconditioning is limited, depending on the variety (Knowles et al., 2009; Kyriacou et al., 2009; Schippers, 1975). Knowles et al. (2009) reported that a reconditioning at 16 °C had a lower effect in reducing RS accumulation in tubers of the 'Umatilla Russet' variety compared to 'Ranger Russet' and 'Russet Burbank' varieties. In another study, Kyriacou et al. (2009) reported that in varieties accumulating high RS levels, the potential of reconditioning to decrease RS is low and suggests using the RS accumulation level and the chipping performance of potato tubers stored at low temperatures (<5 °C) during the first 30 days of storage to evaluate the potential of a reconditioning of potato varieties.

The storage duration needs to be considered as well, since it is well known that potato tuber ageing leads to an irreversible senescent sweetening (Isherwood & Burton, 1975). Driskill, Knowles, and Knowles

(2007) studied the effect of a reconditioning on three potato varieties and showed that even if the reconditioning improved the processing quality for all the tested varieties, the potential of reconditioning was decreased for one cold sweetened variety due to earlier onset of irreversible senescent sweetening over 230 days of storage.

In addition, the time required for reconditioning to avoid sprouting is important to consider and depends of the variety as the dormancy length is variety-dependent (Visse-Mansiaux et al., 2022).

Finally, the weather of a given season should also be considered as an important factor impacting potato quality during storage. It has been reported that climatic conditions during the growing season can influence the physiological age of tubers at harvest and the sprouting during storage (Visse-Mansiaux et al., 2022). Tubers planted later during the season have also been reported to display an increased sucrose content compared to tubers planted earlier (Driskill et al. 2007). In addition, it was recently reported that the sucrose levels can be significantly higher in potatoes grown during the winter season as compared to those grown during the summer season (García-Ríos et al., 2023). Consequently, the initial stock of sucrose might differ depending on the climatic conditions of the growing season and should be taken into account when assessing the dynamic of quality in potatoes processed after storage.

5. Conclusion

Three CIS-resistant varieties have been characterized (*i.e.* Lady Claire, KB and Verdi), that are suitable for storage at low temperature conducive to sprout reduction (*i.e.* 4 °C) with limited sweetening and low acrylamide content for up to four months of storage.

After two months of storage at low temperature, CIS-resistant varieties exhibited high crisp quality, while the three CIS-susceptible varieties displayed poor crisp quality and similar trends were observed after four months of storage. After four months of storage at 4 °C, low acrylamide content was observed in crisps of CIS-resistant varieties, while high acrylamide content was observed in crisps of CIS-susceptible varieties.

Correlation between high transcript levels of *VInv* and high conversion of sucrose to glucose at low temperature has been observed in tubers of the CIS-susceptible Markies variety after four months of storage. Similar correlation has been observed in the CIS-susceptible Agria variety, which had high transcript levels of *VInv* and high glucose content after two months of storage at 4 °C. These correlations were not observed in the CIS-susceptible Pirol variety, meaning that other mechanisms are responsible for RS accumulation in CIS-susceptible varieties, such as the increase of sucrose synthase occurring at low temperature (Duplessis et al., 1996; Pressey & Shaw, 1966; Sowokinos, 2001; Stitt & Sonnewald, 1995).

The CIS-resistance of Verdi and LC varieties can be explained by low transcript levels of *VInv* observed after two months of storage. Surprisingly, the CIS-resistant KB variety had high levels of the *VInv* transcript, meaning that the production of the invertase enzyme has probably been blocked by invertase inhibitors (Greiner et al., 1999) or that there is a rapid reconversion of the reducing sugars produced to sucrose. It would be of interest to measure the kinetic of sucrose "stock" during storage in different varieties in further studies.

Finally, the Markies CIS-susceptible variety responds positively to reconditioning up to 15 °C at four months of storage allowing a decrease of the transcript levels of *VInv* and consequently, a reduction in glucose accumulation and in acrylamide content. It would be of interest to test and screen more varieties for their response to reconditioning.

Our study has helped in the identification of CIS-resistance in potato varieties and in the characterization of the genetic and enzymatic mechanisms involved in CIS.

It is of high importance to determine the ability of several potato varieties to tolerate cold storage without sweetening to expand potato storage management strategies and to use cold temperatures to reduce sprouting instead of using anti-sprouting chemicals.

6. Author statement

All authors listed contributed to the work and are in agreement with the manuscript's content.

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CRediT authorship contribution statement

Margot Visse-Mansiaux: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Leonard Shumbe:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yves Brostaux:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **Theodor Ballmer:** Methodology, Investigation, Conceptualization. **Inga Smit:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Brice Dupuis:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Hervé Vanderschuren:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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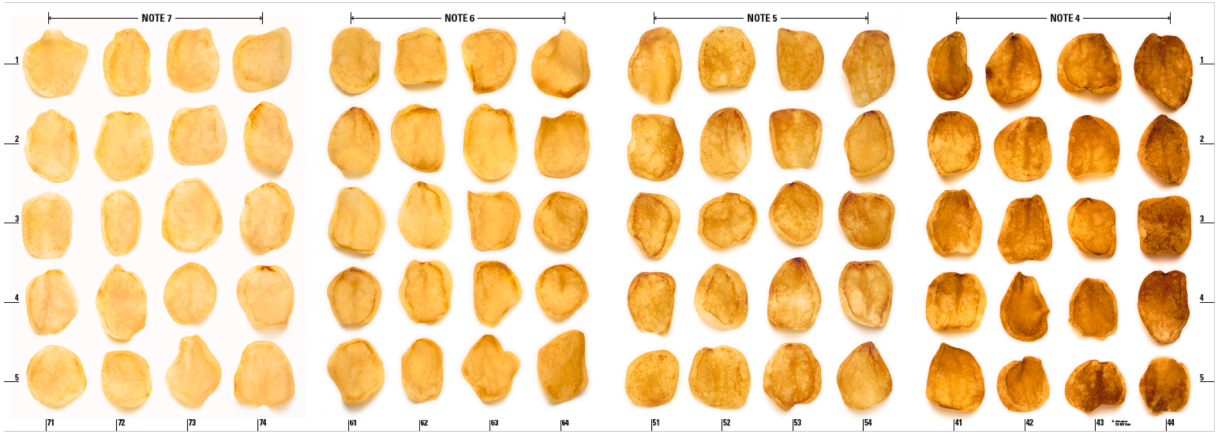
Appendix

Extract of the color scale for quality evaluation of crisps (Swisspatat scale, Edition 2013, Swiss Potato Commission CH-3186 Düdigen, retrieved from: https://www.patate.ch/).



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