Cold sweetening diversity in Andean potato germplasm from Argentina

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ABSTRACT

BACKGROUND: Cold-induced sweetening (CIS) is the accumulation of sucrose and reducing sugars in potato tubers at low temperatures. This process is central for the potato processing industry. During potato chip and French fry production, reducing sugars participate in the Maillard reaction to produce dark pigmented products not acceptable to consumers. Andean potatoes (*Solanum tuberosum* Group Andigena)

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constitute an enormous wealth of potato germplasm that can contribute to increase genetic diversity in breeding programs of many traits, including CIS.

RESULTS: We analyzed reducing sugar content and chip quality in freshly harvested and cold stored tubers from 48 native accessions. Andean accessions showed high variation in reducing sugar content and were classified in three types of CIS responses, I- Reducing sugar content before and after 4 ºC storage was lower than the value required by industry; II- reducing sugar content before storage was acceptable, but after 4 ºC storage incremented up to non-acceptable levels; and III- reducing sugar content was unacceptable before and after storage.

CONCLUSION: Five Andean accessions presented acceptable reducing sugar content and good chip quality before and after 4 ºC storage in a consistent manner throughout several experiments. These features make them a useful source for improving potato industry.

KEYWORDS

Cold sweetening; Andean potatoes; reducing sugars; chip color

INTRODUCTION

Potato (*Solanum tuberosum*) is a native crop from South American highlands that has become the most consumed non-grain food product worldwide (http://faostat3.fao.org). In order to maintain continuous supply of potato throughout the year, potato tubers are often stored at low temperatures. Without cold storage, potatoes have a shelf life of about six months after which their quality rapidly deteriorates.[1] Cold temperature reduces sprouting, losses due to dehydration and respiration, and the spread of diseases. One undesirable consequence of cold storage (< 10 ºC) is a phenomenon named cold-induced sweetening (CIS), in which reducing sugars (glucose and fructose), mainly derived from sucrose hydrolysis accumulate in the tubers.[2] CIS is an hereditable trait [3] and has been explained as an adaptive response to stress caused by cold temperatures.[4] Reducing sugars negatively influence potato chip and French fry quality, as they react during processing with amino acids in the Maillard reaction,[5] resulting in brown- to black-colored food products that are not acceptable to consumers.[2] Additionally, Maillard reaction also generates acrylamide a neurotoxin and a possible human carcinogen.[6] Therefore, preventing reducing sugar accumulation during tuber storage is of economic and nutritional importance to the potato processing industry and one of the main objectives of breeding programs in the world.[7]

Solanum tuberosum Group Andigena comprises native cultivars derived from domestication and selection by Andean farmers ancestrally in the past 7000 years. They are cultivated under short day conditions along the Andes at heights ranging from 2,000 to 4,000 msnm.[8-9] In Argentina, potato landraces are grown in Northwestern provinces (NWA), mainly in Jujuy, Salta and Catamarca.[10] Group Andigena comprises highly polymorphic tetraploid genotypes[9, 11] that show variation in their growth habitat, flower color and tuber characteristics. The high diversity of this Group was demonstrated by studies with morphological descriptors and molecular markers[9, 12-15] constituting an enormous wealth of potato germplasm that can contribute to increase genetic diversity in breeding programs of many traits, including CIS.

In this work we have analyzed 48 accessions of potato varieties from *S. tuberosum* Group Andigena in relation with CIS response. Each accession correspond to a single, vegetative propagated clone maintained by the Active Germplasm Bank of INTA-EEA Balcarce that were collected from different locations in Jujuy and Salta provinces of Argentina. They were selected based on their genetic polymorphism, previously analyzed with set of microsatellites.[14]

The aim of this three-year study was to characterize CIS in Andean germplasm through analyzing reducing sugar content and chip quality of freshly harvested and cold stored tubers.

MATERIALS AND METHODS

Plant material

A total of 48 accessions (*Solanum tuberosum* Group Andigena) deposited in the Active Germplasm Bank of INTA-EEA Balcarce (BAL), Argentina were used (Table 1). Plant material from *in vitro* collection was sown in counter season in a greenhouse for tuber multiplication. Three field trials in randomized blocks with two replications were conducted from November to April of the following year in three consecutive seasons (2010-2011, 2011-2012 and 2012-2013). The first two trials were planted in an experimental field in Humahuaca (23° 12′ 5″ S, 65° 21′ 0″ O, 2939 MAMSL), Jujuy, Argentina, and the third one in a field located in Yavi Department (22° 6′ 4″ S, 65° 35′ 44″ O, 3377 MAMSL), Jujuy, Argentina. We planted 30 accessions in the first field trial increasing the number of accessions through seasons, as they become available, to end up with 48 accessions in the last trial.

Environmental conditions of this region (NW of Argentine) are favorable for Andean tubers production. Local potato cultivation procedures were used.

After harvesting, all tubers were maintained at room temperature until processing (ca. 20 ºC). Then, 10 healthy tubers from each accession were selected and stored at 4 ºC at high relative humidity in the dark. Phenotypic characterization was performed before (freshly harvest) and after two months of storage at 4 ºC. Three healthy tubers of each condition were selected for the analysis, sliced in half from apical end to basal bud and then processed to 1.5-2 mm thickness. The slices were frozen in liquid nitrogen and stored at -80 °C.

Extraction and quantification of reducing sugars

Reducing sugar content was determined as described by Ohara-Takada *et al.*,[16] For reducing sugar extraction we used a sample of three frozen slices (one from each tuber). The sample (ca. 5 g fresh weight) was homogenized with an Ultra-Turrax disperser (IKA, Germany) for 5 min in 15 mL 80 % ethanol, and sugars in the homogenate were extracted at 80 °C for one hour. The extract was filtered through four layers of gauze, and then centrifuged at 10,000 g at 4 °C for 20 min. The supernatant was filtered by Strata Sax 55 μm, 70A column (Phenomenex, EEUU) and a 0.2 μm membrane filter (Microclar, Argentina). Glucose and fructose content were determined by high performance liquid chromatography (HPLC HP, EEUU) with an Amide-80 column (Phenomenex, Luna 5u NH2 100A, 250 x 4.60 mm 5 micron, EEUU). The mobile phase was 80 % acetonitrile/water, and the pump was set at a flow rate of 1 mL min^{-1}. Quantification of reducing sugar was performed by standardization with 0.50 mg mL^{-1} external glucose and fructose (Merck, Germany).

Preparation of potato chips and chip color determination

Twelve fresh slices (four from each tuber) were used for chip production. Tuber slices were water rinsed for 2 min and the surface was dry off with paper towels. Tuber slices were fried at 180 ºC for 2 min or until bubbles were not observed in the oil. Chips were drained and placed on a white surface for observation. Chip color was visually determined with the color cards developed by the Institute for Storage and Processing of Agricultural Produce (Wageningen-The Netherlands), being a score of 9 the lightest and 1 the darkest.

Data analysis

The correlation analysis and means calculi were performed using Prims 5 for Windows software version 5.01. Values were expressed as the means ± standard deviation (SD). In all cases, the confidence coefficient was set at 0.05. Correlation analysis was used to establish the relationship between reducing sugars and chip quality scores. All mean comparisons were carried out using analysis of variance (ANOVA) with Infostat Software (2003 version, www.infostat.com.ar).

RESULTS AND DISCUSSION

To study CIS response we used tubers harvested from three field trials. Reducing sugar content was quantified in two conditions: before (freshly harvested tubers) and after 4 ºC storage for two months. Interaction between reducing sugar content and location/year was not significant ($p>0.1$); therefore we have used the average values of all field trials. Native accessions showed high variation in reducing sugar content of both freshly harvested and 4 ºC stored tubers (Fig. 1). Freshly harvested tubers presented an overall reducing sugar average of 2.08 ± 1.23 g kg^{-1} FW, while after 4 ºC storage, this value was significantly incremented to 6.64 ± 3.49 g kg^{-1} FW. Different accessions showed reducing sugar content in 4 ºC stored tubers varying from 1.09 to 16.65 g kg^{-1} FW (Fig. 1).

Although critical values for fried products differ according processing company standards, it is accepted that reducing sugar content over 2.5 g kg^{-1} FW are not acceptable for the industry.[4, 17-18] Particularly, for chip preparation minor values are desired, since chips are more susceptible to the problem of high reducing sugar concentration than French fries.[19] CIS response in the Andean accessions before and after storage can be classified in three types of responses (Fig. 1**)**. Samples from 5 accessions showed a type I response, in which reducing sugar content before and after 4 ºC storage were below 2.5 g kg^{-1} FW. Thus, accessions CL 641, CCS 1350, CL 650, CCS 1384 and CCS 1199 can be considered as CIS resistant. Twenty six accessions showed a type II response, were reducing sugar contents before storage was acceptable, but after 4 ºC storage reducing sugar contents incremented up to non-acceptable levels. Finally, 17 accessions had a type III response, were reducing sugar levels were higher than 2.5 g kg^{-1} FW before and after 4 ºC storage

We evaluated chip quality from freshly harvested and 4 ºC stored tubers by their color according to a 9 to 1 scale (Institute for Storage and Processing of Agricultural Produce; Wageningen, The Netherlands). The chips industry regards card 5 as showing the darkest acceptable color.[20] Freshly harvested tubers produced light colored chips, with an average score of 7. When tubers were stored at 4 ºC chip quality decreased and the average score fell to a value of 3.8 (Fig. 2). Different accessions showed great color diversity before and after 4 ºC storage with scores that ranged from 1 to 8. Among them, accessions CCS 1330, CCS 1199, CCS 1384 and LC 348 produced good quality chips with scores of 8 even after 4 ºC storage (Fig. 3). Chip color evaluation was difficult in accessions CL 658 and CCS 1385 due to their pink and violet flesh, respectively. In these cases, we only used reducing sugar content to evaluate the CIS response, and these accessions are not included in Figure 2.

Several reports indicate that reducing sugar content is the most important factor governing color of processed product.[21] In this study, there was a significant ($p<0.001$) negative relationship between chip color score and reducing sugar content since the scale uses high scores for light and low scores for dark colors. The correlation coefficients of chip color scores with reducing sugar content in freshly harvested and 4 ºC stored tubers were -0.63 and -0.69, respectively. These values were within the range of correlation values (0.47-0.92) previously reported.[22-23] Regressions of reducing sugar content with chip color scores were explained by exponential functions in both conditions, freshly harvested (R^2=0.41) and 4 ºC stored tubers (R^2=0.55) (Fig. 4).

Seven accessions showed chip color of 7 or above after cold storage (Fig. 2). Four out of the five accessions that presented a type I response, were included in this group, presenting CL 641 a color score after storage of 6. The four most CIS resistant Andean accessions evaluated by chip color were CCS 1330, CCS 1199, CCS 1384 and LC 348 presented scores of 8 after storage (Fig. 2 and 3). Interestingly, CCS 1330 and LC348 -grouped in type II response-presented an average reducing sugar content of 2.63 and 3.48 g kg-1 FW after storage, respectively (Fig. 1). These results, evidence that although, chip color can be explained in a large extent by reducing sugar content, can also be affected by other compounds as polyphenols, amino acids (lysine, glycine, glutamine and arginine) and proteins present in potato tubers[24] that were not determined in this study. Also, color score is a subjective measurement that can be improved by automatic determinations using colorimeters.[25]

Several reports determine sucrose content -the substrate of invertases for glucose and fructose by hydrolisis- as a potential of accumulation of reducing sugars during storage of tubers.[22-23, 26-28]Also, McCann *et al*.,[23] reported significant correlations between sucrose and chip color in some accessions of *S. pinnatisectum*, which supports the hypothesis that hydrolysis of sucrose may occur during frying and that these newly created reducing sugars contribute to chip darkening. However, another work demonstrated that invertase gene silencing caused higher accumulation of sucrose and lower amounts of glucose and fructose resulting in better quality chips than untransformed controls during cold storage.[29] In our work, sucrose concentrations varied widely among the accessions. Mean sucrose concentrations in fresh harvested tubers ranged from 0.75 to 7.77 g kg^{-1} FW, similar to cold stored tubers, which varied from 0.88 to 7.9 g kg^{-1} FW. Likewise, we have not found significantly correlation between sucrose content and chip color. Since sucrose does not react directly in the Maillard reaction[5] it is expected that sucrose concentration has little or no correlation with chip color.

CONCLUSIONS

The use of native and wild germplasm has been proposed as a source of CIS resistance to improve chip quality[23, 26-28] This work, the first characterizing CIS in a large number of Andean accessions, showed a wide variety of responses for this phenomenon. Several accessions presented acceptable reducing sugar content and good chip quality before and after 4 ºC storage in a consistent manner throughout several experiments comprising different years and locations. These features make them a useful source for improving potato using both, conventional breeding or through the aid of molecular techniques.

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Table 1. Accessions planted in field trials. Accessions were provided by Active Germplasm Bank of INTA-EEA Balcarce (BAL), Argentina.

Accessions analyzed	Province	Department	Locality
CCS 1350 Blanca alargada	Jujuy	Tumbaya	El Angosto
CS 1418 Chaqueña	Jujuy	General Belgrano	Papachacra
CS 1432 Collareja redonda	Jujuy	General Belgrano	Cuevas
CCS 1172 Moradita redonda	Jujuy	Tilcara	Casa Colorada
CCS 1251 Chacarera	Jujuy	Cochinoca	Cochinoca
CCS 1330 Moradita	Jujuy	Cochinoca	Rachaite
CCS 1307 Moradita	Jujuy	Santa Catalina	Cabreria
CCS 1166 Cuarentona colorada	Jujuy	Tilcara	Casa Colorada
CS 1419 Blanca	Jujuy	General Belgrano	Papachacra
CCS 1199 Tuni blanca	Jujuy	Humahuaca	Palca de Aparzo
CCS 1349 Colorada	Jujuy	Tumbaya	El Angosto
CCS 1185 Tuni morada	Jujuy	Humahuaca	Aparzo
CCS 1201 Azul	Jujuy	Humahuaca	Varas
CS 1430 Cuarentona	Jujuy	General Belgrano	Cuevas
CL 658 Santa Maria	Jujuy	Yavi	Yavi
CCS 1283 Waicha	Jujuy	Yavi	Yavi
CCS 1295 Rosada	Jujuy	Santa Catalina	Casira
CCS 1371 Chacarera	Jujuy	Cochinoca	Quebraleña
CCS 1205 Churqueña	Jujuy	Humahuaca	Varas
CCS 1247 Tuni blanca	Jujuy	Cochinoca	Ojo de Agua
CL 621 Chorcoyeña	Salta	Santa Victoria	Nazareno
CCS 1271 Blanca	Jujuy	Santa Catalina	Morco Esquina
CCS 1284 Sani	Jujuy	Yavi	Yavi
CCS 1366 Overa	Jujuy	Tumbaya	El Moreno
CCS 1327 Bayista	Jujuy	Cochinoca	Rachaite
CCS1381 Runa	Jujuy	Tumbaya	Patacal
CL 728 Cuarentona	Salta	Iruya	Colanzulí
CL 482 Rosada	Salta	Santa Victoria	Rodeopampa
CCS 1383 Pera o señorita	Jujuy	Tumbaya	Patacal
CCS 1323 Colorada	Jujuy	Cochinoca	Agua Caliente
CCS 1303 Yuruma	Jujuy	Santa Catalina	Casira
CCS 1374 Moradita	Jujuy	Cochinoca	Agua Castilla
CL 631 Allo	Salta	Iruya	Campo Carreras
CCS 1384 Corbatilla	Jujuy	Tumbaya	Patacal
CCS 1385 Moradita	Jujuy	Tumbaya	Patacal
CL 516 Chorcoyeña	Salta	Santa Victoria	Chorro

CL 650 Colorada	Salta	Santa Victoria	Trigohuaico
CL 576 Runa	Salta	Santa Victoria	Lizoite
CL 641 Runa	Salta	Santa Victoria	Poscaya
CL 835 Airampia	Jujuy	Valle Grande	Santa Ana
CL 790 Overa	Jujuy	Valle Grande	Santa Ana
CCS 1170 Ojos colorados	Jujuy	Tilcara	Casa Colorada
CL 836 Airampia	Jujuy	Valle Grande	Santa Ana
CL 708 Runa	Salta	Iruya	Colanzulí
CL 820 Negra redonda	Jujuy	Valle Grande	Santa Ana
CCS 1309 Blanca redonda	Jujuy	Santa Catalina	Cabreria
CCS 1224 Collareja	Jujuy	Humahuaca	Coctaca
LC 348 Imilla negra	Jujuy	Humahuaca	Huachichocana

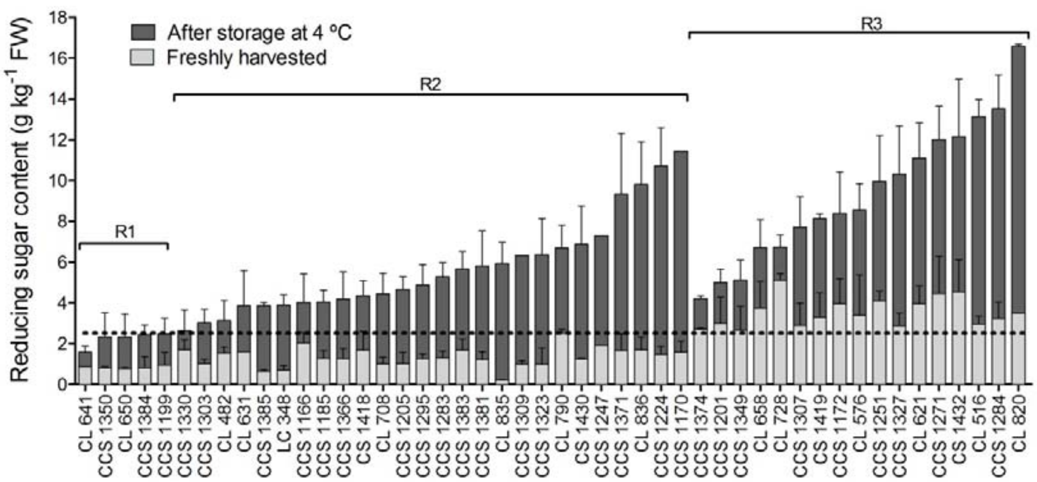

Figure 1. Reducing sugar content (g kg^{-1} FW) and cold sweetening responses (R1, R2 and R3) of freshly harvested (clear bars) and cold stored tubers (dark bars). Bars show mean values and lines ± standard deviations. Dotted line at 2.5 g kg^{-1} FW indicates highest reducing sugar content acceptable by industry.

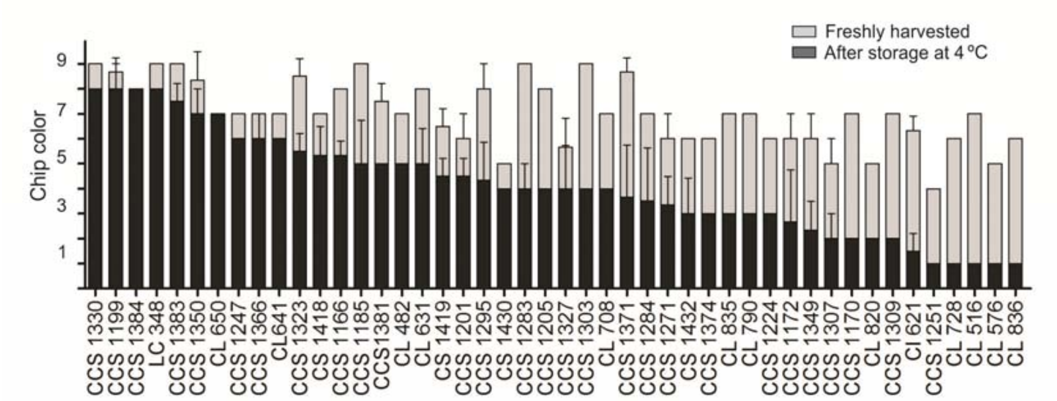

Figure 2. Chip color means from freshly harvested (clear bars) and cold stored (dark bars) tubers. Tubers were stored at 4°C for two months. Chip color was visually determined with color cards developed by the Institute for Storage and Processing of Agricultural Produce (Wageningen-The Netherlands), where a score of 9 is lightest and 1 is darkest.

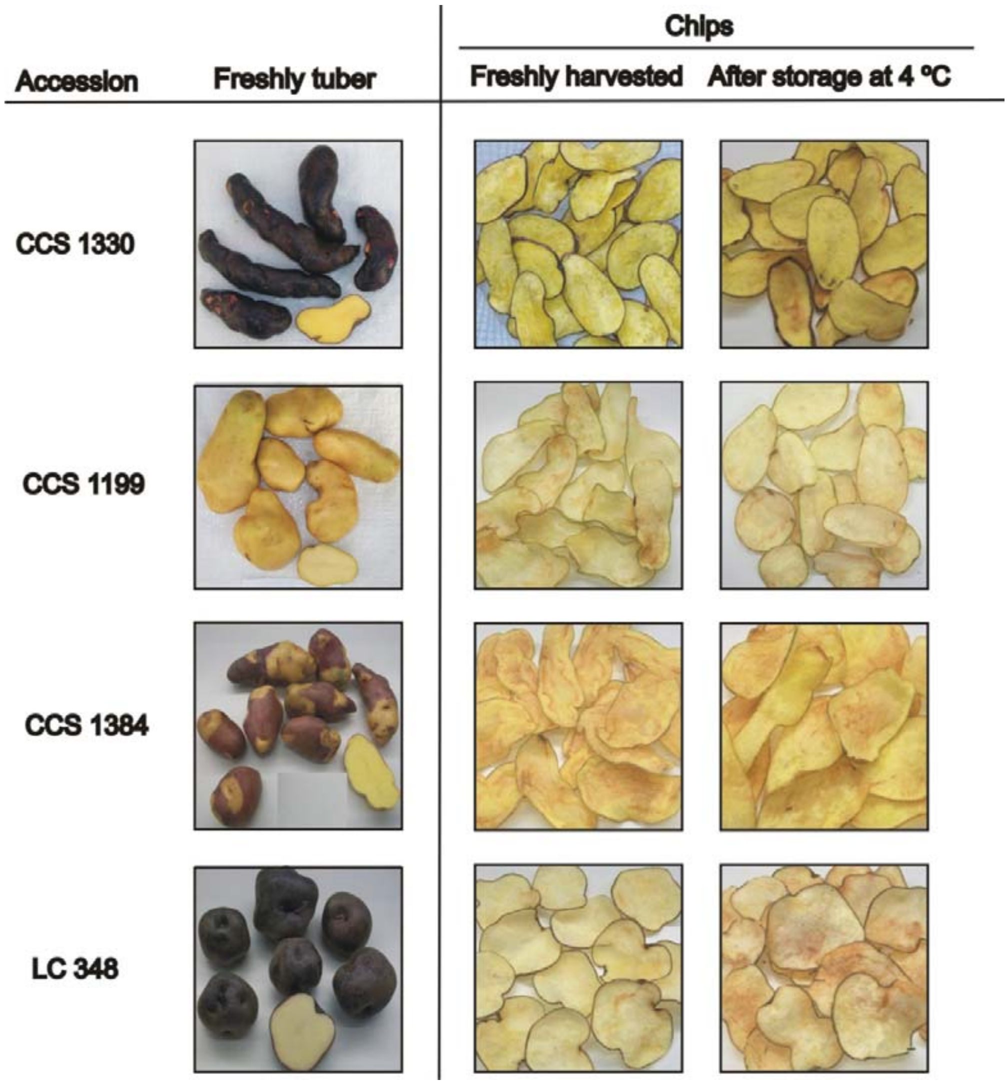

Figure 3. Chips from freshly harvested and cold stored tubers of cold sweetening resistant accessions evaluated by chip color score.

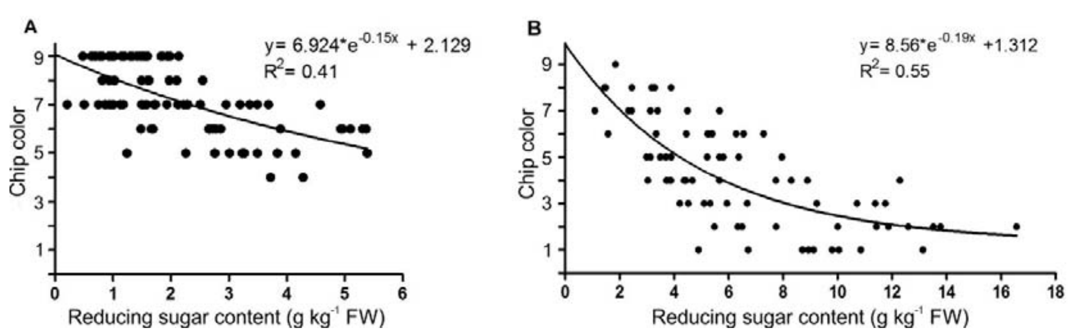

Figure 4. Relationship between reducing sugar content and chip color in freshly harvested (A) and cold stored tubers (B). Regression coefficient (R) and model are showed.