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Effects of Storage Temperature and Duration on Carbohydrate Metabolism and Physicochemical Properties of Potato Tubers

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Abstract

This study aimed to determine the effect of combinations of storage conditions, including storage temperatures ranging from 2 to 22 ºC and storage duration ranging from 3 to 9 days, on potato invertase, sucrose phosphate synthase (SPS), α-amylase, reducing sugar, sucrose, fried chip colour, and fried chip acrylamide. It was found that invertase activity increased when potato tuber was stored at 2 ºC. Duration of 3 - 6 days was needed to activate the invertase. As a result, reducing sugar markedly increased at about 2.5 - 3.8 times. Sucrose also markedly increased due to the low temperature, but it looked like only 2 ºC induced the SPS and resulted in sucrose increase. Low temperature did not affect the α-amylase.

Keywords: Cold-Inducing Sweetening; Invertase; Sucrose Phosphate Synthase; Reducing Sugar; Acrylamide

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Introduction

Potato is the largest vegetable crop in Canada. Approximately 75% of potatoes harvested in Canada are used to produce potato chips, French fries or other processed products. Long term of potato storage is essential to ensure a continuous supply of raw potato materials for Canada's processing industries. Potato storage is a high-risk business which requires significant investment and effective management. It could be possible to potentially lose a significant amount of the crop due to the inappropriate storage conditions and/or the inefficient controls of diseases or insects during potato storage.

Cold-induced sweetening (CIS) is a well-known phenomenon occurring in potato tuber when potato is stored at low temperature in range of 2 - 4 °C. Such low temperature range normally causes an increase in reducing sugar in potato tuber, which will undergo a Maillard reaction with free amino acids and result in a high level of acrylamide in potato frying products at temperature above 120 °C. High level of acrylamide results in unacceptable brown or dark-brown products with a bitter taste. Moreover, it is widely considered as a neurotoxic compound and a probable human carcinogen (Notardonato, et al. 2013) [1]. Generally, reducing sugar is regarded as a limiting factor which determines the degree of acrylamide formation as its amount is much less than the free amino acid amount (Amrein, *et al.* 2003) [2]. Therefore, it becomes crucial to avoid accumulation of reducing sugars during potato storage.

In CIS procedure, starch degradation occurs primarily through the action of starch phosphorylases, in which about 18 enzymes are involved. One of major resources from which reducing sugar comes is the hydrolysis of sucrose, which is the first free sugar to accumulate as a result of starch degradation during sweetening process. Sucrose phosphate synthase (SPS) catalyzes biosynthesis of sucrose-6-phosphate from UDP-glucose and fructose-6-phosphate. Then, a specific phosphatase immediately dephosphorylates sucrose-6-phosphate to free sucrose (Mares, et al. 1985) [3]. The sucrose is hydrolyzed by acid invertase into one molecule of glucose and one of fructose. The phosphorlytic procedure of carbohydrate metabolism in potato tuber were summarized by Sowokinos (2001) [4]. It was reported that storage temperature had no effect on phosphorylase activity (Kennedy and Isherwood, 1975) [5]. However, low temperature significantly increases invertase activity. In addition of the phosphorlytic procedure, α-amylase, β-amylase, and α-glucosidase in the potato tuber also involve the hydrolysis of starch chain, resulting in a mixture of dextrin, maltose, and glucose. α-amylase, β-amylase, and α-glucosidase activities were reported to be higher in tubers stored at 4 °C than those stored at 10 °C (Cochrane, *et al.* 1991) [6]. Sowokinos (2001) [4] figured out that starch degradation is principally phosphorlytic rather than hydrolytic in nature.

Unlike the seed potato, which can be kept at 3 - 4 °C for long term storage, the processing potato needs to be stored at warmer temperature to avoid CIS. Most commercial storage bins in Canada use 9 - 10 °C for long term storage of processing potato. Although the storage conditions of Russet Burbank, a traditional French fry variety mainly used in Canadian potato processing industry, has been well studied, such information is still limited in other potato cultivars or varieties. The objective of this study is to investigate the effects of storage temperatures and duration on sugar changes and carbohydrate metabolism in potato tubers and identify or differentiate optional storage conditions for different potato cultivars or varieties.

Materials and Methods

Potato materials and treatment protocol

Potatoes (*Solanum tuberosum L.*) (Family: *Solanaceae*), cv. Vigor and Lady Claire were obtained from Grand Forks Farm, Taber, Alberta, Canada (latitude 50°40' North and longitude 112°60' West). Potato tubers were harvested during September and October, 2017, at full maturity and stored in a warehouse of the Grand Forks Farm, in which temperature was about 10 °C and relative humidity (RH) 90%. Tuber samples were collected at the end of January 2018 and transported (about one hour) to Lethbridge Research and Development Centre, Lethbridge, Alberta, Canada, and immediately treated under different temperature storages. The storage trials were carried out at 22 °C, 16 °C, 10 °C, 6 °C and 2 °C, respectively, with RH being constant at 90%. The potato tubers were separately packed in black plastic bags with 3 or 4 holes (5 mm in diameter) per square feet and laid on wooden pallets, which promoted air circulation around the tubers. The temperature and RH of the storage treatments were regularly monitored by a thermo-hygrometer (Rotronic Hygroskop GT, Rotronic AG, Zürich, Switzerland) during the whole experimental course. Potatoes were sampled from different temperature treatments after 3, 6 and 9 days of storage, and then was lyophilized for further tests. The baseline analyses of the original tuber, including invertase, total reducing sugar, SPS, α-amylase, sucrose, glucose, and fructose, were conducted in duplicate in the Food Quality Laboratory at the Lethbridge Research and Development Centre, Lethbridge, Alberta, Canada.

Analysis of invertase activities

The activity of invertase was tested using method of Matsuura-Endo, *et al.* (2004). Invertase was extracted from 100 mg of lyophilized tissue which was weighed into a 2.0 ml snap cap tube. Citric acid buffer of pH = 4.5 was added 2.0 mL and vortexed for

2 min. Then the snap cap tube was centrifuged at 13, 000 rpm for 5 min. A volume of 1.5 mL resultant supernatant was "desalted" using a HiTrap 5ml column of Sephadex G-25 (Amersham Pharmacia, Uppsala, Sweden) which was previously equilibrated in the citric acid buffer (pH = 4.5) to remove small molecules, such as glucose and sucrose. A volume of 2.0 mL eluted sample was collected for the inverstase determination. An inverstase assay kit of EnzyChrom™ (EIVT-100) from BioAssay systems (Hayward, CA) was selected to perform the test, in which sucrose cleaved into fructose and glucose by the invertase at 30 °C. The amount of glucose was determined by a colorimetric (570 nm) method. One unit of invertase activity was defined as the formation of 1 µmole glucose per min at pH 4.5 under the assay conditions. Invertase data was collected in duplicate.

Analysis of SPS activity

SPS activity in each treatment was analyzed by using an Elabscience's SPS Assay Kit (Catalog No: E-BC-K597). About 100 mg frozen dried sample was weighed into a 2 ml snap cap tube.

Extract buffer solution with pH = 7.5 was added at 1 ml. The suspension was vortexed for 2 min. Then the homogenate was centrifuged at 8000 g for 10 min. The clean supernatant was transferred into a new 2 ml snap cap tube. The fructose-6-phosphoric acid was hydrolyzed at 37 °C. Then the Optical density measurement was performed at 290 nm wavelength with 1 cm light path quartz cuvette. One-unit SPS activity was defined as production of 1 µmol sucrose catalyzed by 1 mg SPS per minute at 37 °C. Data was collected in duplicate.

Analysis of α-amylase activity

Alpha-amylase activity in each treatment was analyzed by using Megazyme's Assay Kit. About 1.5 gram frozen dried sample was weighed into a 25 mL flask. Into each flask, 10 mL extraction buffer solution was added. The solution in the flask was incubated at 40 °C for 20 min to extract the α-amylase. The, suspension of 10 mL was centrifuged at 1,000 g for 10 min. Supernatant of 1 mL was transferred to a clean 2 mL snap cap tube and pre-incubated at 40 °C for 5 min. Then, supernatant of 0.2 mL was directly added to the bottom of the tube which contained 0.2 mL amylase HR reagent and incubated at 40 °C for exactly 20 min (from time of addition). At the end of the 20 min incubation period, stopping reagent of 3.0 mL was added and stirred the tube contents vigorously. Absorbance of the solutions and the reaction blank at 400 nm against distilled water was collected. One Unit of α-amylase activity is defined as the amount of enzyme, in the presenceof excess thermostable α-glucosidase, required to release one micromole of p-nitrophenol from BPNPG7 in one minute under the defined assay conditions, and is termed a Ceralpha Unit. Two replicate measurements were done for each sample.

Sucrose content

Lyophilized tissue was weighed 0.5 g and dissolved in 5 ml water in a falcon tube. The solution was shaken at room temperature for 60 minutes. After shaking, the solution was centrifuged at 4, 000 rpm for 5 min. Supernatant was transferred 2.0 ml into a 2 ml centrifuge tube. Centrifuge at 4,000 rpm for 5 minutes and transfer 2.0 ml supernatants into 2 ml tubes. Use 1.0 ml sample solution to conduct the phenol and chloroform purification. Sample solution of 1.0 ml was mixed with (i) 1.0 ml phenol, (ii) 0.5 ml phenol + 0.5 ml chloroform, (iii) 1.0 ml chloroform, and centrifuged at 12,000 rpm for 5 min. The supernatants were transferred into new tubes. Sucrose was enzymatically determined by using Megazyme's K-FRUGL Assay Kit. A 96-well microplate was set up to conduct the assay. Absorbance was taken at 340 nm to determine.

Total reducing sugars

Fresh potato was sliced and homogenized in a blender. One gram of homogenate was weighed out into a 15 ml falcon tube and 10 ml of 33 % ethanol was added and the suspension was shaken linearly for 16 hours. The suspension was centrifuged at 2,800 rpm for 5 minutes, and the supernatant was collected in a 15 ml falcon tube. Reducing sugar (glucose and fructose) was enzymatically determined by using Megazyme's K-FRUGL Assay Kit. 96-well microplate format was selected to conduct the tests. Absorbance was taken at 340 nm to determine D-glucose and D- fructose content. Total reducing sugar was calculated by summing up the D-glucose and D-fructose. Data of total reducing sugar were obtained from six replicates.

Potato Chip Preparation

Potato chips were prepared according methods of Zhang, *et al.* (2018) [8] which some minor modifications. After being stored for 3, 6, and 9 days in the dark at various temperatures, eight medium sized potato tubers were selected, washed and peeled. The tubers were sliced into chips approximately 1.5 mm thick. The slices were rinsed immediately after slicing for approximately 1 min in tap water. Twenty uniform slices, which were 7–8 cm in diameter, were randomly selected and dried with paper towels on both sides. About ten dried chips were fried in canola oil at 180 °C for 3 min in a 3.5 L T-fal fryer (Tefal, Rumilly, Haute-Savoie, France). After frying, the chips were cooled at room temperature, and then stored in plastic bags at - 20 °C for future acrylamide analysis.

Results and Discussion

The untreated fresh tubers were tested for carbohydrate metabolism and physicochemical properties in the laboratories of Cereal & Food Chemistry at the Lethbridge Research and Development Centre, AAFC, Canada. The results are showed in Table 1.

Table 1: Baseline data of Vigor and Lady Claire potato tubers*

	Invertase	R. S.	Sucrose	Total Starch	SPS	α-amylase	Acrylamide
Vigor	10.56±0.31	75.453±8.850	328.03±14.34	60.982±0.129	2.43±0.047	3.2±0.11	3.800±0.39
Lady Claire	3.83±0.192	57.448±5.933	216.75±6.35	70.656±1.019	2.06±0.260	5.2±0.61	4.370±0.18

*Invertase - $\times 10^{-3}$ unit of invertase, dry basis; R. S. - Reducing sugar, mg/100g, fresh basis; Sucrose - %, dry basis; SPS – Sucrose concentration created in the sample per minute at 28 °C at pH=7.4, μmol^{-1} minite^{-1}, dry basis; α-amylase - $\times 10^{-2}$ unit/g min at 40 °C, Cerapha unit, dry base; Acrylamide - mg/1000g chip.

Effects of storage temperature and duration on invertase

The invertase activity results are shown in Figure 1. At 2 °C, Vigor invertase activity increased from 10.42×10^{-3} up to 19.30×10^{-3} and then to 35.26×10^{-3} unit, while Lady Claire increased from 3.83×10^{-3} up to 9.40×10^{-3} and then to 15.55×10^{-3}, when storage duration increased from 3 to 6 and then to 9 days. After being stored at 2 °C for 3 days, Vigor and Lady Claire respectively contain invertase activity of 10.42×10^{-3} and 3.83×10^{-3} unit, which are the same as those of the corresponding parent potato tubers, indicating activation of the invertase was induced by a ≥ 3- day cold treatment, rather than instantaneous simulation. In another words, it was at 4^{th} – 6^{th} day when the decomposition of sucrose occurred after cold storage under 2 °C. At 6 – 10 °C, the invertase activity also increased with storage duration increasing from 3 days to 9 days. However, the increase intensity did not match those under 2 °C. At temperatures of ≥16 °C, Vigor and Lady Claire invertase kept stable during the storage period which were about 12×10^{-3} and 6×10^{-3} unit, respectively. Figure 1 also show that Vigor has higher invertase activity than Lady Claire, indicating Vigor variety has higher potential to produce more reducing sugar than Lady Claire during storage.

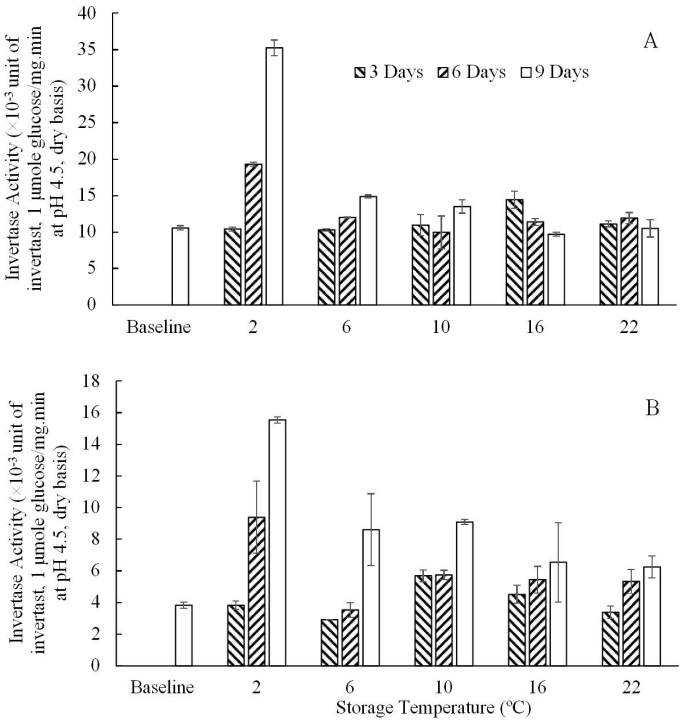


Figure 1: Effect of storage days and temperature on invertase activity. A: Vigor. B: Lady Claire

Effects of storage temperature and duration on SPS

The results were shown in Figure 2. The parent Vigor and Lady Claire respectively have SPS of 2.43 and 2.06 μmol^{-1} minite^{-1}. After stored at 2 °C both Vigor and Lady Claire showed increase in SPS from 3-day to 9-day storage. However, at ≥ 6 °C, Vigor SPS decreased, while Lady Claire SPS just fluctuated At 22 °C, Vigor tuber showed high SPS of about 3.5 μmol^{-1} minite^{-1}. This is probably because high temperature activated SPS in Vigor tubers.

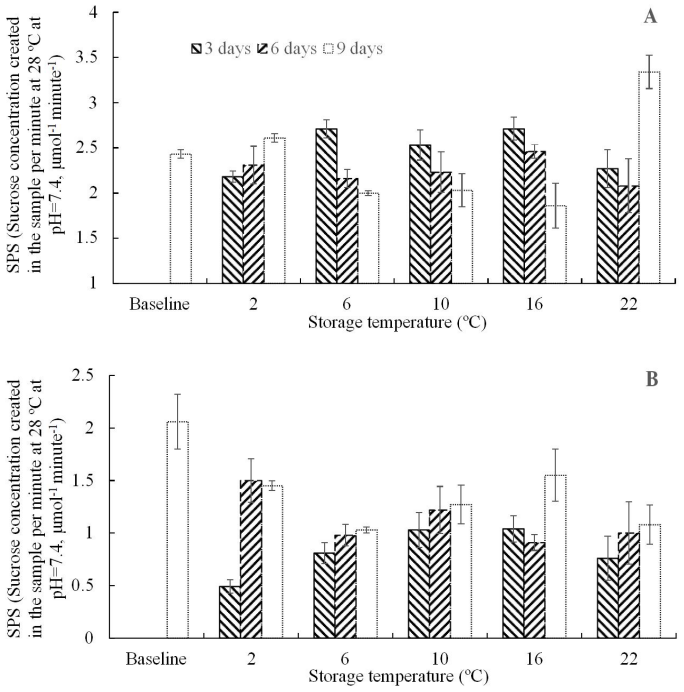


Figure 2: Effect of storage days and temperature on SPS. A: Vigor. B: Lady Claire

Overall, the results shown in Figure 2 indicate only temperature of 2 °C significantly impacted the SPS. Pollock and Rees (1975) [9] claimed that SPS activity did not increased when potato tuber was cooled. Hill, *et al.* (1996) [10] stated that the maximum activity of SPS and the total amount of SPS protein did not change after store potato tubers under 4 °C.

Effects of storage temperature and duration on α-amylase

In order to better understand why sucrose increased at 2 °C, α-amylase activity was tested in this study. The result is shown in Figure 3. The parent Vigor and Lady Claire respectively have α-amylase activities of 3.2 and 5.2×10^{-2} unit/g min. During the storage duration, the α-amylase activities of both varieties increased up to around

8 – 9 × 10^{-2} unit/g min. However, the activity of α-amylase fluctuated with storage temperature and storage duration. Low temperature did not activate α- amylase. Interestingly, Figure 3 shows 2 ºC depressed the α-amylase, an opposite effect on the α- amylase. Cochrane, *et al.* (1991) [4] and Cottrell, *et al.* (1993) [11] observed that α-amylase activity was higher in tubers stored at 4 ºC than those stored at 10 ºC. Nielsen, *et al.* (1997) [12] further indicated that the cold-induced activity in potato tuber is β-amylase. They found when potato tuber was transferred from 20 to 5 and 3 ºC, the ctivity of β-amylase was progressively induced and increased 4- and 5-fold within 3 days of storage at 3 ºC. Our study did ot show the similar response. The reason is unclear We hypothesize that during starch phosphorlytic degradation, low temperature of 2 ºC activated the phosphatase activity which accelerated the reaction of sucrose-6-phoshphate to sucrose and, as a result, accumulated sucrose concentration in the tuber. Further test is needed to verify the hypothesis.

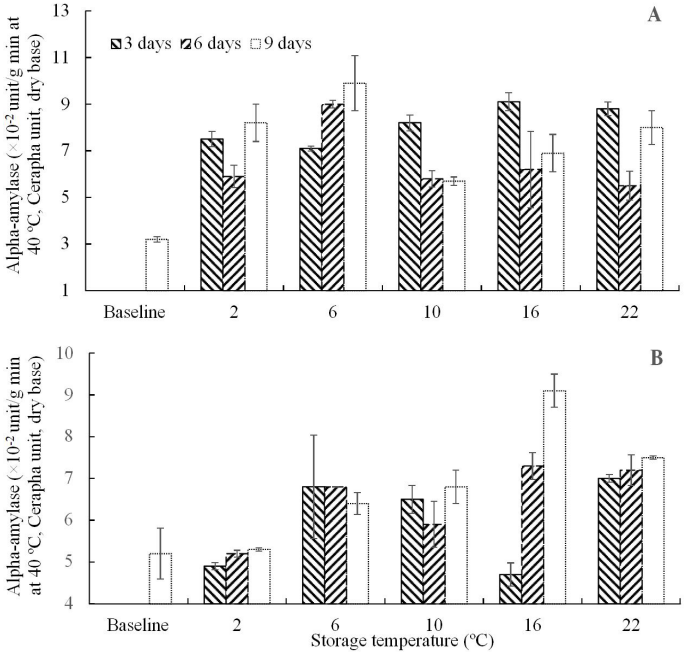


Figure 3: Effect of storage days and temperature on α-amylase activity. A: Vigor. B: Lady Claire

Effects of Storage Temperature and Duration on Sucrose

Sucrose results was shown in Figure 4. The parent Vigor and Lady Claire contain 328.03 and 216.75 % (d.b) sucrose. At 2 ºC, sucrose significantly accumulated for both varieties during the storage duration. Vigor markedly increased from 585.7 to 1244 mg/100g (dry basis, db), while Lady Claire from 440.6 to 738.2 mg/100g (db). According to Mares, *et al.* (1985), sucrose is produced from sucrose-6-phosphate which is bio-synthesized by UDP-glucose and fructose-6-phosphate. SPS is the catalyst which can accelerate the reaction. In Figure 2, the SPS activity was significantly activated by the low temperature of 2 ºC. Like, the Vigor SPS increased from 2.18 to 2.61 mg/100g (db), while Lady Claire 0.49 to 1.45 mg/100g (db). The current sucrose results are consistent with the SPS responses. Krause, *et al.* (1998) [13] stated that cold storage caused shift in the apparent molecular weight of SPS and its kinetic properties. This shift impacted the levels of the hexose phosphates and UDPglucose, leading to sucrose accumulation in cold-stored potato tubers. At 6 ºC, Vigor and Lady Claire sucrose slightly increased. At ≥ 10 ºC, sucrose concentrations remained relatively stable at ranges of 360 – 430 mg/100g (db) and 260 - 460 mg/100g (db) for Vigor and Lady Claire, respectively.

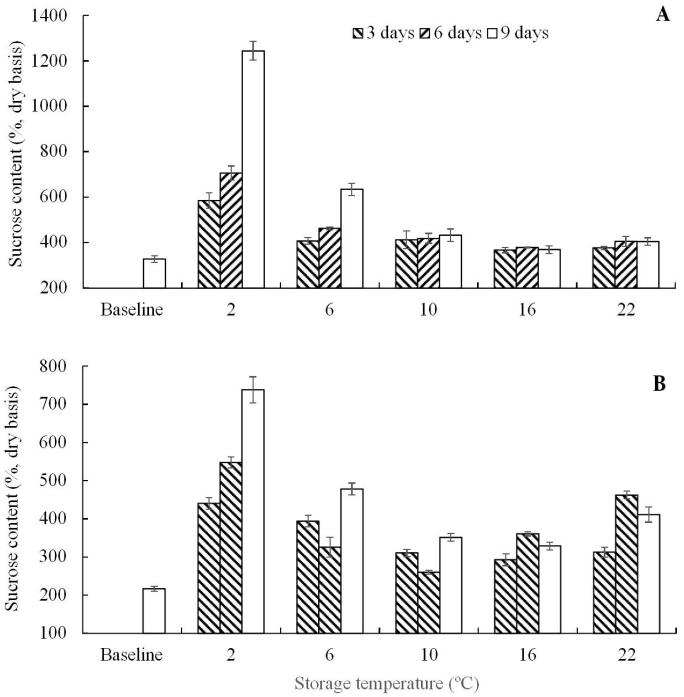


Figure 4: Effect of storage days and temperature on sucrose. A: Vigor. B: Lady Claire

Effects of storage temperature and duration on reducing sugar

Figure 5 shows the reducing sugar levels in different storage temperature and duration. At 2 ºC, reducing sugar in Vigor significantly increased from 48.0 to 184.2 mg/100g of tuber (fresh basis, fb) with storage duration increasing from 3-day to 9-day, while Lady Claire increased from 61.1 to 150.7 mg/100g, indicating "CIS" occurred. This result is consistent with the invertase responses in Figure 1. The parent Vigor and Lady Claire contain 75.453 and 57.448 mg/100g reducing sugar. At 2 ºC, the reducing sugar did not increase until the 6th day when invertase triggered the hydrolysis of sucrose into glucose and fructose. At temperature ≥ 6 ºC, reducing sugar level fluctuated, but generally respectively remained stable at 60 - 80 mg/100g for Vigor and 50 -60 mg/100g for Lady Claire. Li, *et al.* (2013) [14] confirmed that reducing sugar in the potato tubers stored at 4 ºC increased from 150 up to 350 mg/100 with the storage duration increasing

from 15 to 30 days. According to Pedreschi (2007) [15], a limit of reducing sugars ranging from 150 to 200 mg/100 g of tuber (fb) is practically used as an indicator for suitability of potatoes for processing. Figure 6 and Figure 7 show the glucose and fructose contents in the tubers. For Vigor, the average levels of glucose and fructose in tubers stored at 2 °C for 9 days respectively accumulated up to 90.05±7.685 and 88.15±15.854 mg/100g (fb), while, for Lady Claire, they reached up to 77.40±2.781 and 73.34±3.146 mg/100g (fb), respectively. These values are 2 - 3 times as much as those in other conditions. Ohara-Takada, *et al.* (2005) reported that, at 2 °C, glucose and fructose increased gradually and reached approximately 170 mg/100g (fb) after 14 days. Although the amounts of fructose and glucose are similar to each other in the raw tuber, it is commonly recognized that fructose plays larger role in the acrylamide formation. According to Chuda, *et al.* (2003) [16], since the rate of the Maillard reaction depends on the kinds of sugar, the contribution to acrylamide production would differ with the different kinds of sugars.

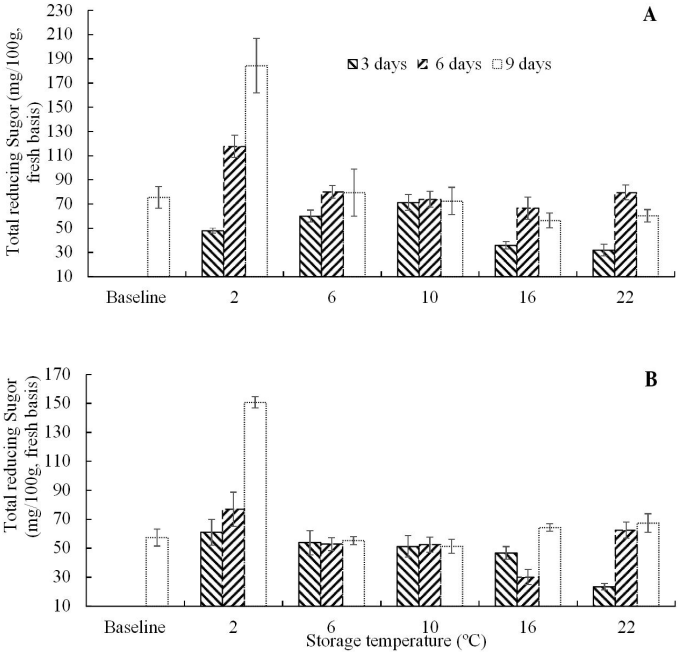
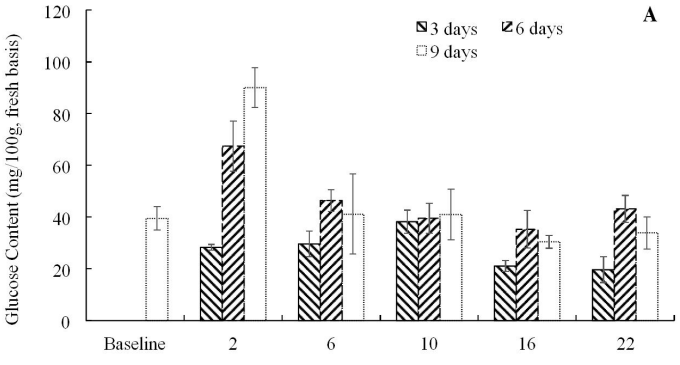


Figure 5: Effect of storage days and temperature on reducing sugar. A: Vigor. B: Lady Claire



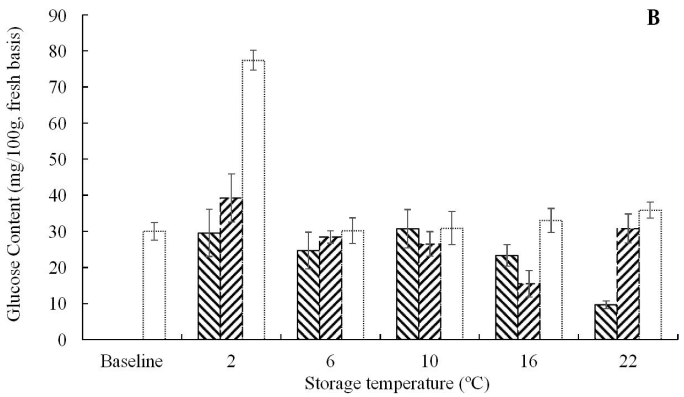


Figure 6: Effect of storage days and temperature on glucose. A: Vigor. B: Lady Claire

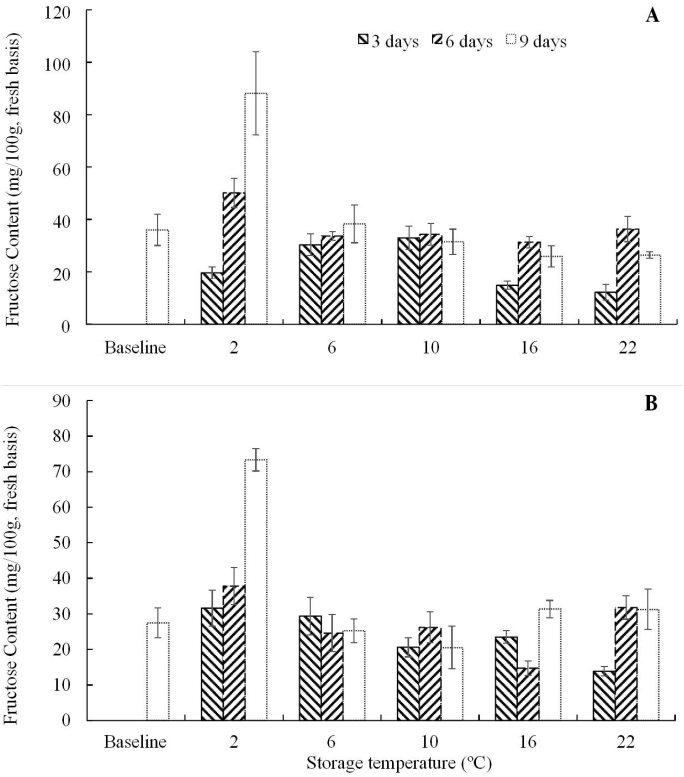


Figure 7: Effect of storage days and temperature on fructose. A: Vigor. B: Lady Claire

Effects of Storage Temperature and Duration on Chip Colour

The visible results of "CIS" is the color of the potato chips. Figure 8 shows the color of the chips with Vigor and Lady Claire respectively stored at 2 and 22 °C for 9 days being representatives. After 9-day storage at 2 °C, the chips were brownish yellow as shown in Figure 6A and 6C, while after 9-day storage at 22 °C, the chips were light yellow as shown in Figure 6B and 6D. This phenomenon was expected and consistent with the invertase response and reducing sugar responses. Since reducing sugar content significantly increased at 2 °C during 9-day storage, in turn increasing acrylamide formation during the frying process, potato chips with brownish-yellow color was resulted.

Figure 8: Appearances of potato chips respectively stored under 2 ˚C and 22 ˚C for 9 days. A: Vigor, 2 ˚C, 9 days. B: Vigor, 22 ˚C, 9 days, C: Lady Claire, 2 ˚C, 9 days. D: Lady Claire, 22 ˚C, 9 days

Conclusion

Storage temperature and duration in the range of 2 - 22 ºC and 3 - 9 days were investigated in this study. The results confirmed that the low temperature storage could definitely induce CIS. Storage conditions of 2 ºC and 9 days markedly increased invertase activity which resulted in significantly increase in reducing sugars 2.5 - 3.8 times. Only very low temperature, such as 2 ºC, increased the SPS activity and resulted in increase in sucrose content. Temperature in range of 4 - 22 ºC did not have influence on SPS. This study did not indicate the low temperature increases the α-amylase activity. This was hypothesize that low temperature of 2 ºC activated a reaction acceleration of sucrose-6-phoshphate to sucrose, which accumulated sucrose concentration in the potato tuber. Colour measurement and appearance observation confirmed the CIS occurred in low temperature condition. The chips made from 2 ºC exhibited to be brownish yellow. In this study, vigor tuber contained significantly higher content of sucrose, glucose and fructose than Lady Claire because it was more sensitive to cold stress and accumulated more sugars under low temperature storage. Lady Claire was more sensitive to hot stress and accumulated more sugar during high temperature, etc. Tuber sugar changes under different storage conditions could be attributed to genotype-dependency.

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