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Effect of Storage Temperature on Carbohydrate Metabolism and Development of Cold-Induced Sweetening in Indian Potato (*Solanum tuberosum* L.) Varieties

Galani Yamdeu Joseph Hubert.,[1,*] Pooja H. Gupta,[2] Nilesh J. Patel.,[2] Avadh K. Shah[2] and Jayant G. Talati.[2]

[1]Department of Agriculture and Veterinary Medicine, Université des Montagnes, P.O. Box 208, Bangangté, Cameroon

[2]Department of Biochemistry, B.A. College of Agriculture, Anand Agricultural University, Anand-388110, Gujarat, India

[*]Corresponding author: Galani Yamdeu Joseph Hubert

Telephone: +237674244181

Fax number: +237222239802

E-mail address: josephgalani@gmail.com

Abstract

This study investigated the changes in carbohydrate metabolism in tubers of 11 Indian potato varieties stored at room temperature, 15ºC and 4ºC for 150 days to understand the development of cold-induced sweetening (CIS). Low temperature storage negligibly influenced starch and maltose contents of tubers, but induced a significant increase of reducing sugars, total soluble sugars, fructose, glucose, hexoses:sucrose ratio and decrease of sucrose content was noticeable at 4ºC. A strong positive correlation was found between reducing sugars and total soluble sugars, and between fructose and glucose. The activity of β-amylase was considerably increased by storage at low temperature, and it weakly correlated with starch content. Also, absence of maltose accumulation with increased β-amylase activity was observed. Acid invertase activity drastically rose at low temperature and strongly paralleled reducing sugars, glucose, fructose and hexoses:sucrose ratio. Variety K. Jyoti was designated as CIS-tolerant and K. Badshah as CIS-susceptible.

Keywords: Cold-induced sweetening; Potato; HPLC; Sugars; Acid invertase; β-amylase

Practical Applications

Development of cold-induced sweetening (CIS) is important for basic research and for potato processing industry. This work allowed to group 11 Indian potato varieties into low sugar-forming and high sugar-forming groups, to identify varieties suitable for processing immediately after harvest or short time storage, to identify varieties with high starch content suitable for starch extraction. Hence it provides capital information to industrial about varieties with good starch yield, that can be cold stored without drastic sugar increase; and to breeders for searching genes of resistance to CIS in Indian potatoes. This study also demonstrated that during CIS development in these varieties, acid invertase is the key enzyme, β-amylase is not the main enzyme of starch degradation and there is possible significant activity of maltase in potato tubers. These observations pave the way through biotechnology work to develop new potato varieties which can cope with this post-harvest problem.

1. Introduction

Potato (*Solanum tuberosum* L.) is the third most important food crop, the most important non-grain food crop and one of the most essential basic vegetable worldwide as well as in Indian subcontinent. After harvest, potatoes are stored in cold conditions to provide round the year supply to markets and consumers (Marwaha *et al*., 2010). But during storage at cold temperatures, many cultivars accumulate free reducing sugars derived from breakdown of

starch to sucrose that is ultimately cleaved by acid invertase to produce glucose and fructose in a metabolic process known as cold-induced sweetening (CIS). It is a widespread phenomenon that has long been recognized (Menendez *et al*., 2002) and is explained as a shift in the balance between starch degradation and glycolysis, leading to the accumulation of sucrose (Suc) which is then converted into glucose (Glu) and fructose (Fru) (Isherwood, 1973). The reducing sugars (RS) have been implicated in frost tolerance by serving as cryoprotectants (Stitt and Hurry, 2002). However, when cold-stored potatoes are processed, Glu and Fru participate in the Maillard reaction with free amino acids (arginine) during frying or dehydration at high temperature, resulting in dark-brown-coloured and bitter tasted fries and chips. These darkened chips and fries are unacceptable to consumers and also result in greater amounts of acrylamide production which has been linked to many cancers (Chuda *et al*., 2003; Hogervorst *et al*., 2007).

Processing quality of potato is determined by large size of tubers, high dry matter and low RS. Low RS (<250 mg/100 g tuber fresh weight) and high dry matter (>20%) are basic requirements for the preparation of good quality fried potato products like chips or French fries and dehydrated products like flakes, flour and powder (Ezekiel *et al*., 2003). The amount of sugars increases in cold stored potatoes due to the increased activities of carbohydrate splitting enzymes. Many enzymes along the pathway of carbohydrate metabolism in potato tubers have been investigated as possible control points for CIS. However, the activities of acid invertase (INV) (Matsuura-Endo *et al.,* 2004; McKenzie *et al.,* 2005) and β-amylase (BAM) (Nielsen *et al.,* 1997; Karim *et al*., 2008) play a major role in accumulation of hexose sugars. Correlations between INV activity and the hexoses:sucrose ratio have been made in cultivars exhibiting varying resistance to CIS (Zrenner *et al*.,1996; Matsuura-Endo *et al*., 2004; McKenzie *et al*., 2005). Furthermore, Brummell *et al*. (2011) suggested that endogenous potato invertase inhibitors, if present in particular cultivars could potentially be important in determining the extent of CIS.

Currently, number of potato breeding programs are searching for potatoes varieties resistant or tolerant to CIS. Cultivars resistant to CIS would provide several economic benefits, such as less need for sprout inhibitors, reduced dry matter loss, easy maintenance of high relative humidity, less pathogen problems during storage and less chance of chilling injury during harvest, storage and transit (Burton 1969; Duplessis *et al*., 1996). Therefore, varieties with low RS after cold storage are required to meet the growing demand of the processing industry. Unfortunately, despite extensive breeding efforts, no truly CIS-resistant cultivars have been released onto the market, and CIS remains one of the major issues facing the potato-processing industry (McKenzie *et al*., 2013). Moreover, there is less information available on development of CIS in Indian potato cultivars (Kumar, 2011), hence the present work was planned.

2. Materials and methods

2.1. Plant materials

Tubers of 11 potato (*Solanum tuberosum* L.) Indian cultivated varieties were obtained from Main Vegetable Research Station, Anand Agricultural University, Anand. They included DSP 287, DSP 186, Kufri Surya, Kufri Chipsona-3, Kufri Sutlej, Kufri Sadabahar, Kufri Jyoti, Kufri Lauvkar, Kufri Himsona, Kufri Bahar and Kufri Badshah. Healthy and uniform potato tubers of the 11 cultivars grown according to recommended package were collected after harvest. The tubers were cured during 15 days at room temperature to allow starch sugar conversion, wound healing or lesion tuberization. The tubers were then separately packed in small plastic net bags and stored in different conditions i.e., (i) at room temperature (25-32ºC), (ii) in the incubator (15ºC) and (iii) in cold storage (4ºC). Samples were collected for analysis at 0, 7, 15, 30, 45, 60, 75, 90 and 105 days of storage, i.e. a total of 9 samplings. However, samples at room temperature were collected up to 60 days only, because of decaying. For each sampling period, 3 tubers of each variety were randomly taken and thoroughly washed with tap water. The peal was removed and immediately the flesh was cut into small dices of approximately 0.5 cm^3 and pooled. The pooled sample was powdered with liquid nitrogen using a mortar and pestle.

2.2. Estimation of reducing sugars

The dinitrosalicylic acid (DNS) method described by Sadasivam and Manickam, (2007) with some modifications was used. Sugars were extracted from 100 mg of the sample in 10 mL of 80% (v/v) methanol for 2 h on an orbital shaker. The solution was centrifuged at 13,000 g for 10 min and the methanol was evaporated from the supernatant by keeping it on a water bath at 80°C for 25 min. After cooling, extracted sugars were dissolved by adding 10 mL of distilled water in the tube. Aliquot of 2 mL extract was taken and 3 mL of DNS reagent (prepared by dissolving 1 g DNS, 200 mg crystalline phenol and 50 mg sodium sulphite in 100 mL 1% NaOH) were added and tubes were heated in a boiling water bath for 5 min. When the contents of the tubes were still warm, 1 mL of 40% Rochelle salt (Potassium sodium tartrate) solution was added and after cooling at room temperature, 10 mL of distilled water was added and absorbance was read at 510 nm. Glucose standards (100 to 500 µg) were prepared and the amount of reducing sugars present in the sample solution was calculated using the standard graph and expressed in mg/100 g fw.

2.3. Determination of starch

The procedure of Kuan *et al*., (2011) was used with some modifications. Ten ml of distilled-deionised water (ddH_2O) was added to 0.1 g of potato powder and incubated in a water bath (80°C) with regular shaking for 30 min. The slurry was then centrifuged at 13,000 g for 10 min

at 4°C. The residue was washed 8 times with 40 mL of ddH_2O for removing soluble carbohydrates, the resultant precipitate was oven-dried at 80°C for 8 h, and 2 ml of ddH_2O was added and hydrolyzed at 100°C in 2 ml of 6 N HCl for 15 min. After cooling the Glu content was estimated by phenol sulphuric acid method as described by Sadasivam and Manickam, (2007). The sample solution (0.1 mL) was taken and volume made to 1 mL with ddH_2O water, then 1 mL of 5% phenol solution followed by 5 mL of 96% sulphuric acid were added to each tube and shaken well. After 10 min the contents in the tubes were shaken and placed in a water bath at 25–30°C for 20 min. After cooling at room temperature, absorbance was read at 490 nm. Glucose standards (20 to 100 µg) were prepared and the Glu content of the sample solution was calculated using the standard graph. The value was multiplied by a factor 0.9 to obtain the starch content expressed in percentage of fresh weight tuber (% fw).

2.4. Estimation of total soluble sugars

For estimation of TSS, 0.2 mL from the RS extract (see section 2.2) was taken and sugars were quantified by phenol sulphuric acid method as described in section 2.3.

2.5. Sugars profiling through High Performance Liquid Chromatography

Glucose (Glu), fructose (Fru), sucrose (Suc) and maltose (Mal) contents were determined by high-performance liquid chromatography (HPLC) analysis. Potato powder 0.5 g was homogenized in 1 mL of 80% (v/v) methanol; the slurry was incubated in water bath at 85°C for 15 min, cooled and centrifuged at 13,000 g for 10 min. The supernatant was filtered through a 0.22 µm Polyvinylidene fluoride (PVDF) membrane filter before injection. A Waters Acquity UPLC™ H Class System equipped with a Quaternary Solvent Manager, a Sample Manager FTN and a Refractive Index Detector model 2414 was used to analyze the sugar signals. A Waters HPLC Amino (NH_2) Carbohydrate Analysis column (10 µm, 3.9 mm x 300 mm) was operated at 35°C. The injection volume was 20 µl, the isocratic elution mobile phase was an acetonitrile and water solution (80:20) at a constant flow rate of 1.8 mL/min. Detector cells temperature was 35°C with sampling rate 2 and sensitivity 32. Sample Manager's temperature was maintained at 20°C. Each sample was injected thrice and the running time was 10 min. Sugar standards (Glu, Fru, Suc, and Mal) were individually prepared by dissolving 10 mg sugar into 1 mL of water and used for identification and quantification. Limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting a series of dilute solutions with known concentrations on the basis of a signal-to-noise ratio equal to about 3 and 10, respectively. The linearity standard curves were obtained by 3 injections of standards (Glu, Fru, Suc and Mal) mixture at 50, 100, 500, 1000 and 2500 ppm. The output signal was monitored and processed using Empower 3 Software; peaks were identified by comparing retention times

with sugar standards and peaks areas were calculated automatically. To minimize errors due to intra-day and inter-day variations in retention times, several bracketings were performed with a mixture of standards at 1000 ppm. Peaks with signal-to-noise ratio less than 10 were considered below the quantification limit (BQL). The content of each sugar was calculated against standard curves and expressed in mg/100 g fw. The hesoses (Glu+Fru):sucrose ratio was then calculated.

2.6. Enzymes activities

2.6.1. Extraction of carbohydrates splitting enzymes

Potato powder (1g) was homogenized in 1.5 mL of ice-cold extraction buffer containing 50 mM 4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid (HEPES)-KOH, pH 7.3, 15 mM $MgCl_2$, 2 mM ethylene diamine tetraacetic acid (EDTA), 1 mM ethylene glycol tetraacetic acid (EGTA), 2 mM dithiothreitol (DTT), 10% (v/v) glycerol, and 2% (w/v) polyvinyl polypyrrolidone (PVP). The crude extract was centrifuged at 14 000 g for 20 min at 4°C and the supernatant was collected. Protein concentration of extracts was determined according to Lowry *et al*. (1951).

2.6.2. β-amylase activity

The activity of β-amylase was determined using a protocol adapted from Kuan *et al*. (2011). Protein extract (0.5 mL) was added to 1.5 mL sodium acetate buffer (0.016 M, pH 4.8) containing 1% soluble starch, and incubated in a water bath (37°C) for 15 min. The reaction was stopped by the addition of 2 ml of DNS reagent, heated for 5 min in boiling water and cooled, followed by the addition of 10 ml of 4% Rochelle salt solution. Maltose standards (200 to 1000 µg) were prepared. The absorbance at 510 nm was measured and the maltose content of reaction mixture before and after incubation was tested. Then BAM activity was expressed as gram of maltose produced per min (one unit) per g fw (g Mal/min/g fw or U/g fw).

2.6.3. Acid invertase activity

The procedure of Brummell *et al*. (2011) was used with some modifications. Enzyme extracts were shaken for 90 min rapidly enough to generate foaming, to minimize the activity of endogenous invertase inhibitor proteins. Enzyme assays contained 200 µL of protein extract and 800 µL of 40 mM sucrose in 60 mM phosphate-citrate buffer, pH 5.0. Reactions were carried out at 30ºC for 30 min, and were stopped by transferring the tubes in water batch (94ºC) for 3 min. Control reactions for each sample were performed by heating the reaction aliquots of protein extract to 94ºC for 3 min before addition of substrate buffer. Glucose formed was determined against a standard curve by DNS method and INV activity was expressed as nmol of Glu formed per hour per mg protein (nmol Glu/h/mg protein).

2.7. Statistical analysis

Experiments were performed in 3 replications. Data obtained were analyzed using a factorial completed randomized design with Analysis of Variance (ANOVA) carried out at 1% and 5% levels of significance to determine the significance of difference among the varieties and storage temperatures for each of the parameters studied. The difference was considered as significant for p-value < 0.05 and as very significant for p-value < 0.01. Pearson's correlation at 1% and 5% levels of significance was also performed between various parameters. The software SPSS Statistics 17.0.3 was used.

3. Results and Discussion

3.1. Reducing sugar content

Initially, the highest RS content (147.56 mg/100 g fw) was obtained from K. Sutlej and the lowest (79.39 mg/100 g fw) from K. Badshah, which was significantly lower than all the other varieties. During storage, changes in RS content significantly varied among varieties and incubation temperatures: the lowest contents were recorded at room temperature and the highest in cold storage. The RS content very significantly increased by storage at 4ºC and the degree of increase varied from one variety to the other. A drastic increase in RS content was observed at 45 or 60 days storage, and at 105 days varieties K. Jyoti and K. Badshah showed the lowest (1790.33 mg/100 g fw) and the highest (2509.85 mg/100 g fw) RS content, respectively. Overall, storage increased RS by 1.5 to 4.4-fold at room temperature, 2.8 to 4.7-fold at 15ºC and 14.7 to 31.6-fold at 4ºC. The most important criterion of RS content for processing potatoes (less than 150 mg/100 g fw) was recorded by most of the varieties at room temperature during the 60 days storage, up to 75 days at 15ºC but only up to 15 days at 4ºC (Figure 1).

These RS content values are comparable to those obtained by other researchers on similar Indian potato cultivars and genotypes (Uppal and Ezekiel, 2002; Meena *et al*., 2009, Kaul *et al*., 2010; Bhardwaj *et al.*, 2011; Kumar, 2011). Also, several authors have reported an increase of RS in cold storage (Uppal and Ezekiel, 2002; Karim *et al*., 2008; Abong *et al*., 2009; Meena *et al*., 2009; Kaul *et al*., 2010; Bhardwaj *et al.*, 2011; Kumar, 2011; Chen *et al.,* 2012; Kumar *et al*., 2012; Ou *et al*., 2013). Besides, varieties with low RS at harvest were not necessarily having low RS after cold storage, indicating that the degree of increase in RS content during storage varied with the genotype (Bhardwaj *et al.,* 2011; Ou *et al*., 2013). The variation in CIS sensitivity among different potato cultivars, which is manifested by differences in their carbohydrate content during storage, is presumably due to a composite of differences in mRNA expression patterns and post-transcriptional events (Brummell *et al*., 2011).

3.2. Total soluble sugar content

The initial TTS content was highest in K. Sutlej (246.48 mg/100 g fw) and lowest in DSP 186 (110.08 mg/100 g fw) which was significantly lower than all the other varieties. Storage increased the TSS content of potatoes, with a very significant difference observed between the 3 storage temperatures. A significant increase was observed at 60 or 75 days in tubers stored at 4ºC and K. Jyoti, K. Lauvkar, K. Himsona showed the highest TSS contents while the lowest value was recorded in K. Badshah. In general, the increase in TSS was 1.8 to 9.9-fold at room temperature, 2.3 to 11.7-fold at 15ºC and 7.7 to 22.2-fold at 4ºC (Figure 2).

These results are similar to the findings of Karim *et al*. (2008) and Chen *et al*. (2012). Incubation of potato tubers at <10˚C causes accumulation of soluble sugars (mainly Suc, Glu and Fru) which is likely to increase the resistance of tissues to cold shock. Increased TSS content might be due to enzymatic hydrolysis of starch to sugar at low temperature (Cochrane *et al*., 1991; Nielsen *et al*., 1997; Karim *et al*., 2008). Hence the lowest increase of TSS observed at 15˚C may result from a low activation of starch degrading enzymes. At last, storage at 15˚C appeared to be the best temperature for maintaining low TSS.

3.3. Starch content

The content of starch originally ranged between 3.37% fw (K. Sutlej) and 8.35% fw (K. Lauvkar). During storage, in general the starch content was lowest at initial day and it increased up to 30 to 45 days of storage, then a decrease was observed. The only significant difference in changes of starch content was found between room temperature and incubator. The increase of RS content coincided with the decrease of starch content. Throughout the storage period, DSP 287, K. Jyoti, K. Himsona, K. Bahar and K. Badshah showed higher starch contents as compared to other varieties (Figure 3).

Potatoes with high starch yield are required by industries for production of starch and others dehydrated products. These results of starch content are in agreements with the findings of Sood *et al*. (2008), Hassanpanah *et al*. (2011) and Li *et al.* (2013). However, during storage a continuous dwindle in the amount of starch was reported by Karim *et al*. (2008) in potatoes cold stored at 0-2ºC for 8 weeks while in this study, starch content increased during initial days and the decrease was observed later. The amount of starch is reduced due to the hydrolysis of starch by starch degrading enzymes. Thus the increase in starch content observed between 0 and 30-45 days in this study can be explained by starch and sugar metabolism oriented towards starch synthesis (Kim and Lee, 1993). In mature dormant tubers, the sugars are produced by degradation of a small fraction of starch (Isherwood 1973). This could justify why the increase of RS content paralleled the decrease of starch content.

3.4. Sugars profiling through HPLC

Quantification of Fru, Glu, Suc and Mal by HPLC revealed that Fru remained very low and most of the time was below quantification limit (BQL) during storage at room temperature (0.8 to 2.8-fold increase) and in incubator (0.5 to 4.1-fold increase). In cold storage however, Fru remained low until 15 days of storage, and then it rapidly increased and was maintained high up to 105 days (21.4 to 85.0-fold increase). At 105 days, the lowest and the highest Fru content (793.30 and 1837.85 mg/100 g fw, respectively) were recorded from DSP 287 and K. Lauvkar, respectively (Figure 4).

Similar trend was observed with Glu, with increase varying from 0.3 to 7.6-fold at room temperature, 0.3 to 11.3-fold at 15ºC and 9.0 to 99.3-fold at 4ºC. At 105 days, the lowest and the highest Glu content (795.51 and 1908.06 mg/100 g fw, respectively) were recorded from DSP 287 and K. Lauvkar, respectively.

Between the first and the last day of storage, Suc increased 1.5 to 5.0-fold at room temperature; it decreased 1.8 to 7.5-fold at 15ºC and the decrease observed from 15-30 to 105 days at 4ºC ranged between 1.5 and 8.9-fold. At 105 days, the lowest and highest remaining Suc contents (142.97 and 606.29 mg/100 g fw, respectively) were obtained from K. Badshah and K. Himsona, respectively (Figure 5).

The results of changes in sugars content after cold storage obtained here are on par with those reported by Karim *et al*. (2008), Kumar (2011), Kumar *et al*. (2012) and Chen *et al.* (2012). On the other hand, our results of Glu contain are in contrast with the findings of Luthra *et al.* (2009) and Kaul *et al*., (2010). Similarly, the Suc contents obtained at 0 day storage are higher than the values reported by Kumar *et al.* (2003) and Pandey *et al*. (2008). These disparities may be attributed to the different varieties used in each study, the storage temperatures or the method used to analyse the sugars. For instance, the results of Luthra *et al.* (2009) were obtained from wild species and Kaul *et al*., (2010) from K. Chipsona-1 and K. Chipsona-2. Moreover these authors measured the sugars enzymatically using a Biochemistry Analyzer or the amount of Suc was deduced from TSS and RS. Here we have analyzed the sugars by HPLC which is known to be a more sensitive and more precise method.

The values of sugars profiling by HPLC and changes in Fru, Glu and Suc after storage in cold conditions obtained in this investigation are in corroboration with the data reported by Vivanti *et al*. (2006) and McKenzie *et al*. (2013). Bhaskar *et al.* (2010) also observed a rapid increase of Glu and Fru after 14 days storage and the decline of Fru and Glu content observed in DSP 287 and K. Surya between 75 to 105 days in this study are kin to the decrease observed from 60 to 180 days by these authors. Conversely, our results are slightly higher than the findings of Ohara-takada *et al*. (2005). It can be observed that the trend of changes in Glu followed those

of Fru. Also, changes in Suc in one side and Glu or Fru on the other side were inversely proportional: a drop in Suc paralleled an increase in Glu and Fru. The amount of Glu and Fru increased in cold stored potato due to the enzymatic conversion of starch and Suc to RS (Nielsen *et al*., 1997; Karim *et al*., 2008). A large proportion of the Suc enters the vacuole (Blenkinsop *et al*., 2004), where it is irreversibly cleaved into Glc and Fru by INV (McKenzie *et al*., 2013).

The Mal content was not essentially affected by storage temperature and the value fluctuated up and down over the course of the entire study. Overall it ranged between 6.89 to 78.90 mg/100 g fw. Kuan *et al*., (2011) reported similar observations during low temperature storage of sweet potato. The absence of Mal accumulation should therefore not be taken as an indication against increased hydrolytic starch degradation. Maltose can be hydrolysed to glucose by the enzyme maltase. Therefore the study of maltase activity in potato tubers could explain the observed variations in Mal content.

3.5. Hexoses:sucrose ratio

The hexoses:sucrose ratio was lowest at room temperature (maximum 0.38), higher at 15ºC (maximum 2.68) and highest at 4ºC (maximum 19.50). It varied in a nonspecific pattern at room temperature and incubator. In cold storage however, it increased during the whole storage period. Also, except at 105 days, K. Jyoti has shown lowest hexoses:sucrose ratio values while highest values were recorded with K. Badshah (Figure 6). These observations are consistent with the analysis of other authors which also found a higher ratio in potato tubers stored at 4ºC (Zrenner *et al.,* 1996; Chen *et al*., 2008). High hexoses:sucrose ratio indicates a high degree of Suc conversion to Glu and Fru. Variability among the cultivars can be explained by differences in INV activity and/or compartmentalisation of sucrose amongst cultivars (Sowokinos *et al*., 1989).

3.6. β-amylase activity

Initial BAM activity (in gMal/min/g fw) ranged between 1.13 (DSP 287) and 0.86 (K. Himsona), which was significantly on par with DSP 186, K. Sadabahar, and K. Jyoti. Storage temperature considerably influenced BAM activity. In general, a decrease of activity or just a slight increase was observed from 0 to 30 days of storage but at 45 days, the activity increased suddenly, followed by a gradual increase until 105 days. The 11 varieties responded differently to different storage temperatures. For the whole storage period, the increase in BAM activity varied from 1.2 to 1.7-fold at room temperature, 2.1 to 3.1-fold at 15ºC and 2.7 to 3.6-fold at 4ºC (Figure 7).

Nielsen *et al.* (1997) and Karim *et al*. (2008) also reported similar folds of increase in BAM activity in potato tubers stored in cold temperatures. It can be noticed that, BAM activity

inversely followed starch content: decrease in starch content coincides with increase in BAM activity, and vice versa (Figures 3 and 7). However, Nielsen *et al.* (1997) observed that this increase in BAM activity was followed by Mal accumulation while no Mal increase was recorded in this study. This may be attributed to high maltase activity in potato varieties used in the present study which might have immediately hydrolyzed most of the Mal formed after BAM activity into Glu.

3.7. Acid invertase activity

Activity of INV was strongly influenced by storage temperature. In all the 3 storage temperatures, the enzyme activity weakly increased from 0 to 15 days, then at 30 days it raised up rapidly followed by a gradual increase till 105 days, with the highest increase recorded at 4ºC. At 105 days of storage at 4ºC, the lowest and the highest INV activity were obtained from K. Jyoti (2.57 nmol Glu/h/mg protein) and K. Badshah (3.97 nmol Glu/h/mg protein), respectively. Between the first and the last day of observation, the activity of INV increased from 7.6 to 39.3-fold at room temperature, 7.6 to 56.7-fold at 15ºC and 20.1 to 144.3-fold at 4ºC (Figure 8).

Higher increase of INV activity in cold-stored potatoes as compared to high temperature-stored potatoes has been largely documented. Our results corroborate the findings of Cheng *et al.* (2004), McKenzie *et al.* (2005), Karim *et al*. (2008) and Liu *et al*. (2011). Moreover, the changes in INV activity followed the changes observed in RS, Glu and Fru contents (Figures 1, 4 and 8). Also, the increased activity of INV is associated with the decreased amount of Suc content: with the low INV activity observed in initial days, Suc content increased; then the sudden increased of INV activity observed from 30 days onwards subsequently lead to a dwindle of Suc content (Figures 5 and 8). Similar trends were also reported by different authors (Karim *et al*., 2008; Bhaskar *et al*., 2010; Zhang *et al*., 2013).

3.8. Correlations between carbohydrate metabolism parameters

Table 1 presents Pearson's correlation coefficients between carbohydrate metabolism parameters, each for 242 observations. The TSS correlated very strongly with RS (R^2=0.901) as well as all other parameters but showed no relationship with Suc (R^2=0.172) and Mal (R^2=0.055). The same trend was observed for correlation between RS and the other parameters. This is in agreement with the observations of Kamrani *et al*. (2011) who also found a very strong correlation (R^2=0.914) between TSS and RS. This implies that RS share the highest contribution to the TSS content.

The contents of Fru and Glu had an almost perfect relationship with each other (R^2=0.989) and a very strong relationship with TSS and RS. However, Mal did not correlate with any parameter.

These results confirm the significant contribution of Glu and Fru to the increase of sugars observed during CIS and the possible degradation of Mal trough other pathways.

Although a negative relationship was expected between BAM and starch, surprisingly no relationship (R^2=0.058) was found. However, a strong relationship was obtained between BAM and TSS (R^2=0.603) or RS (R^2=0.733). Zhang *et al*. (2013) also reported a similar correlation (R^2=0.861) between BAM and RS. This disparity can be explained by the fact that except BAM, other enzymes such as starch phosphorylases play critical role in the process of starch degradation in potato tubers during cold storage (Zeeman *et al*., 2004; Rommens *et al.,* 2006; Kamrani *et al*., 2011). Also, glucan water dikinase (GWD) is a key enzyme controlling the phosphorylation degree of starch and therefore shares a synergistic effect in BAM-mediated starch degradation (Mikkelsen *et al*., 2005; Edner *et al*., 2007).

As expected, the correlation was found very strong between INV and Glu (R^2=0.860) and Fru (R^2=0.861) as well as with TSS (R^2=0.825) and RS (R^2=0.869), and was strong with the hesose:sucrose ratio (R^2=0.696). Zhang *et al*. (2013) also reported a similar correlation (R^2=0.732) between hesose:sucrose ratio and INV while Ou *et al*. (2013) observed a lower association (R^2=0.343) between the two parameters. The RS content in a potato tuber is synergistically controlled by several carbohydrate metabolism-related enzymes (Sowokinos 2001) and our results support that INV activity is crucial for the process of CIS (Ou *et al*., 2013).

4. Conclusion

From the above findings, it can be concluded that on the basis of their high starch content maintained during storage, DSP 287, K. Jyoti, K. Himsona, K. Bahar and K. Badshah are the most proper varieties for starch production. Considering their low sugar content, all the studied potato varieties appeared to be suitable for processing immediately after harvest or short storage at room temperature but after long term low-temperature storage, they showed high amounts of sugars, but K. Jyoti, K. Surya, K. Sutlej, K. Sadabahar and K. Himsona can produce better quality of fried products as their sugar increase was acceptable. Based on the degree of sugar increase during storage, reflected by the hexoses:sucrose ratio, RS content and INV activity, the 11 potato varieties could be grouped as low sugar-forming varieties (K. Jyoti, K. Surya, K. Sutlej, K. Sadabahar and K. Himsona) and high sugar-forming varieties (DSP 287, DSP 186, K. Chipsona-3, K. Lauvkar, K. Bahar and K. Badshah). Therefore, K. Jyoti has been selected as CIS-tolerant and K. Badshah as CIS-susceptible. None of the varieties used in this study was found resistant to CIS which remains one of the serious hurdles of potato processing industry.

Consequently, there is an urgent need to develop new varieties capable to cope with this cold stress.

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Figure legends

FIGURE 1.

CHANGES IN REDUCING SUGARS CONTENT OF POTATO VARIETIES STORED AT THREE DIFFERENT TEMPERATURES.

Sugars were extracted in 80% methanol and reducing sugars content was estimated using the dinitrosalicylic acid method.

FIGURE 2.

CHANGES IN TOTAL SOLUBLE SUGARS CONTENT OF POTATO VARIETIES STORED AT THREE DIFFERENT TEMPERATURES.

Sugars were extracted in 80% (v/v) methanol and total soluble sugars content was quantified by phenol sulphuric acid method.

FIGURE 3.

VARIATIONS OF STARCH CONTENT OF POTATO VARIETIES STORED AT THREE DIFFERENT TEMPERATURE.

Soluble carbohydrates were washed from potato tubers powder, remaining starch was hydrolyzed with 6N HCl at 100°C and glucose released was estimated by phenol sulphuric acid method. The value of glucose content was multiplied by a factor 0.9 to obtain starch content.

FIGURE 4.

CHANGES IN FRUCTOSE CONTENT OF POTATO VARIETIES STORED AT THREE DIFFERENT TEMPERATURE.

Sugars were extracted in 80% methanol, filtered through a PVDF membrane and 20µL were separated through an Amino (NH_2) HPLC column at 1.8 mL/min flow rate of acetonitrile:water (80:20) mobile phase. Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times of standards and fructose content was calculated according to peak area.

FIGURE 5.

CHANGES IN SUCROSE CONTENT OF POTATO VARIETIES STORED AT THREE DIFFERENT TEMPERATURE.

Sugars were extracted in 80% methanol, filtered through a PVDF membrane and 20µL were separated through an Amino (NH_2) HPLC column at 1.8 mL/min flow rate of acetonitrile:water (80:20) mobile phase. Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times of standards and sucrose content was calculated according to peak area.

FIGURE 6.
DIFFERENCE IN HEXOSES:SUCROSE RATIO OF POTATO VARIETIES STORED AT THREE DIFFERENT TEMPERATURES.
Sugars content was determined by HPLC and the ratio was calculated as sum of Glucose content + Fructose content divided by Sucrose content.

FIGURE 7.
CHANGES IN BETA-AMYLASE ACTIVITY OF POTATO VARIETIES STORED AT THREE DIFFERENT TEMPERATURES.
Enzymes were extracted in Tris-acetate buffer, potato starch was used as substrate and activity of β-amylase was measured as maltose formed after 15 min of reaction.

FIGURE 8.
CHANGES IN ACID INVERTASE ACTIVITY OF POTATO VARIETIES STORED AT THREE DIFFERENT TEMPERATURES.
Enzymes were extracted in HEPES-KOH buffer, sucrose was used as substrate and acid invertase activity was measured as glucose formed after 30 min of reaction.

Table 1. Correlations coefficients between carbohydrates metabolism parameters.

	Starch	**TSS**	**RS**	**Suc**	**Fru**	**Glu**	**Mal**	**H:S Ratio**	**BAM**	**INV**
Starch	1.000									
TSS	0.036**	1.000								
RS	-0.019	0.901	1.000							
Suc	0.064**	0.172	-0.058	1.000						
Fru	-0.034**	0.880	0.944	-0.038**	1.000					
Glu	-0.026*	0.875	0.938	-0.021**	0.989	1.000				
Mal	-0.057	0.055**	-0.008**	0.023**	-0.010**	-0.020**	1.000			
H:S Ratio	-0.039**	0.603	0.754	-0.246	0.781	0.777	-0.022**	1.000		
BAM	0.058**	0.682	0.733	-0.272	0.657	0.679	0.003**	0.594	1.000	
INV	0.142**	0.825	0.869	-0.087	0.861	0.860	0.045**	0.696	0.832	1.000

*, **: significant at 5% and 1% level, respectively

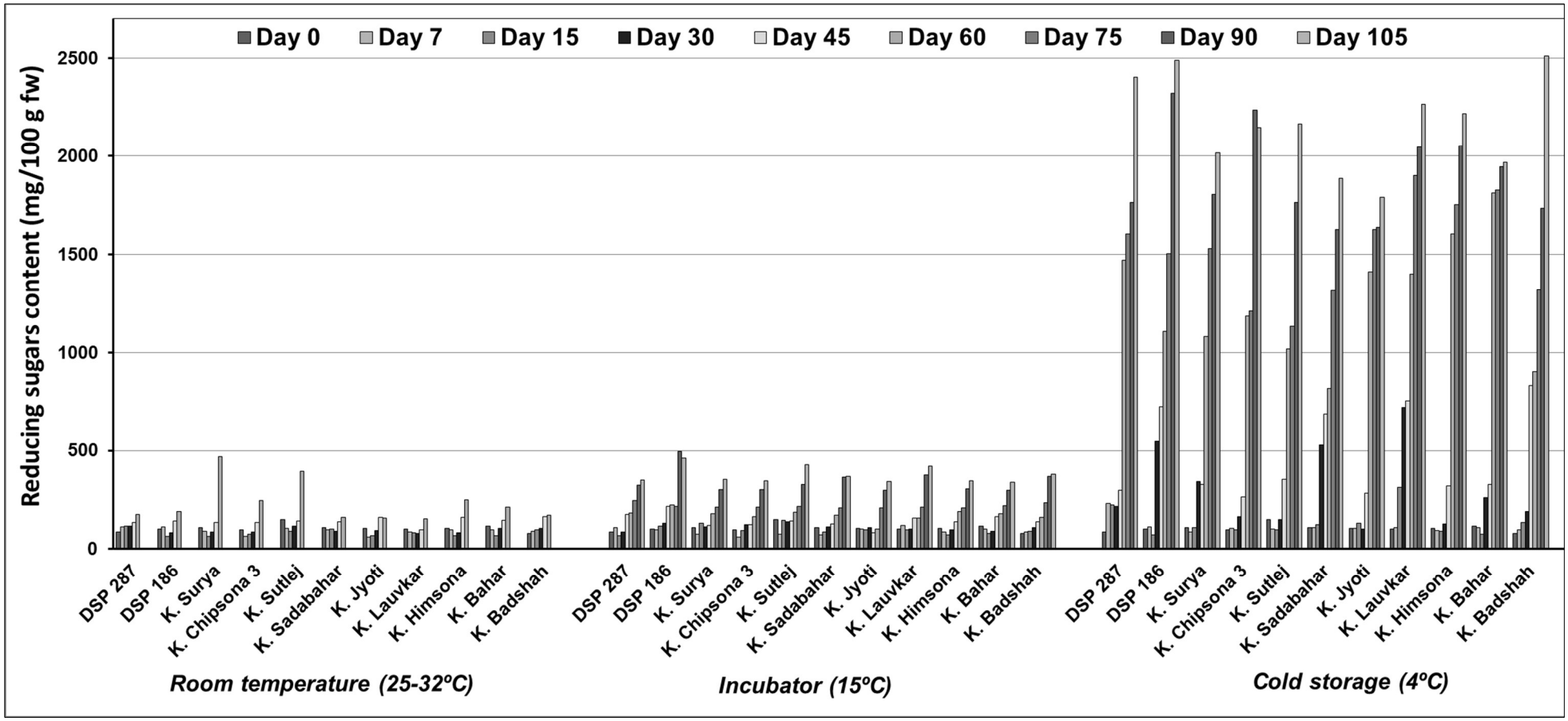

Figure 1. Changes in reducing sugars content of potato varieties stored at three different temperatures. Sugars were extracted in 80% methanol and reducing sugars content was estimated using the dinitrosalicylic acid method.

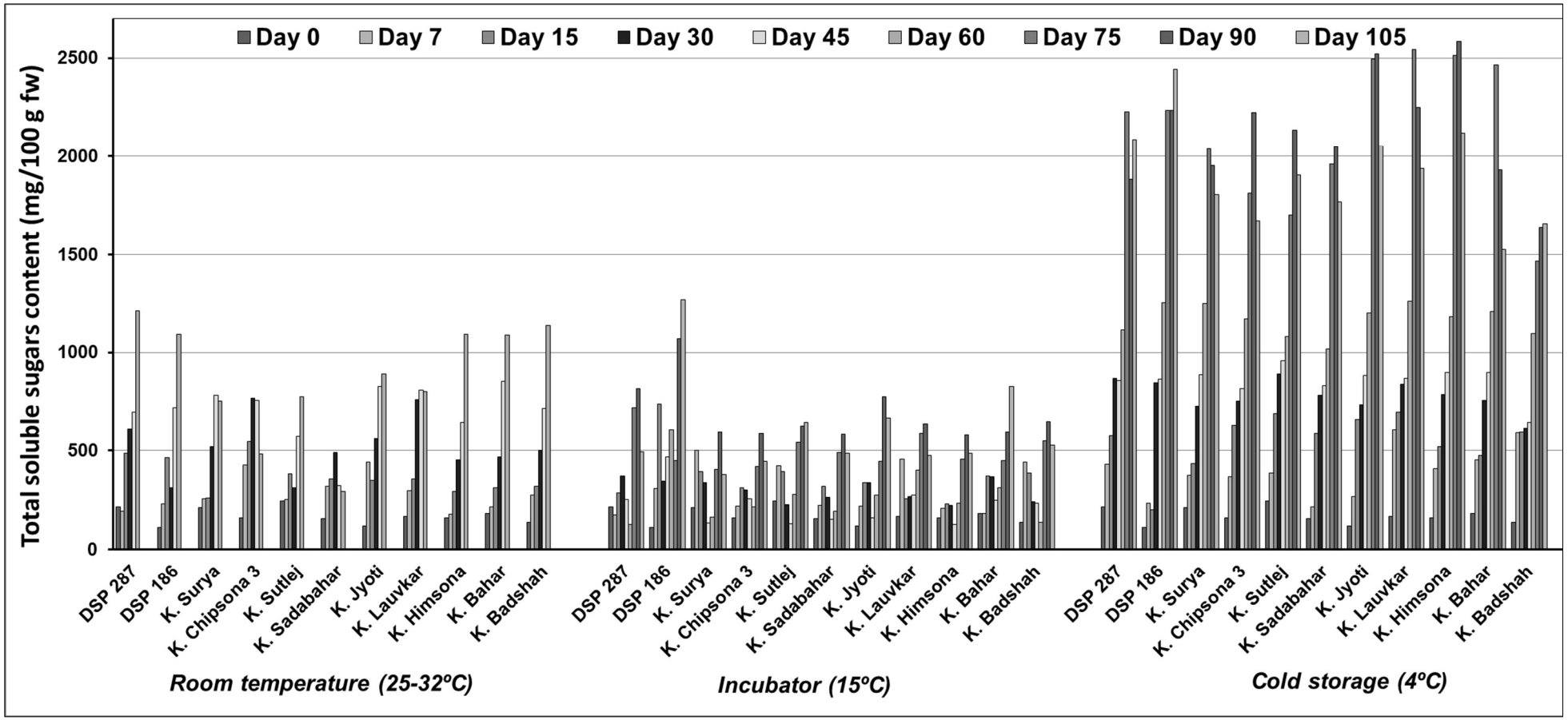

Figure 2. Changes in total soluble sugars content of potato varieties stored at three different temperatures. Sugars were extracted in 80% (v/v) methanol and total soluble sugars content was quantified by phenol sulphuric acid method.

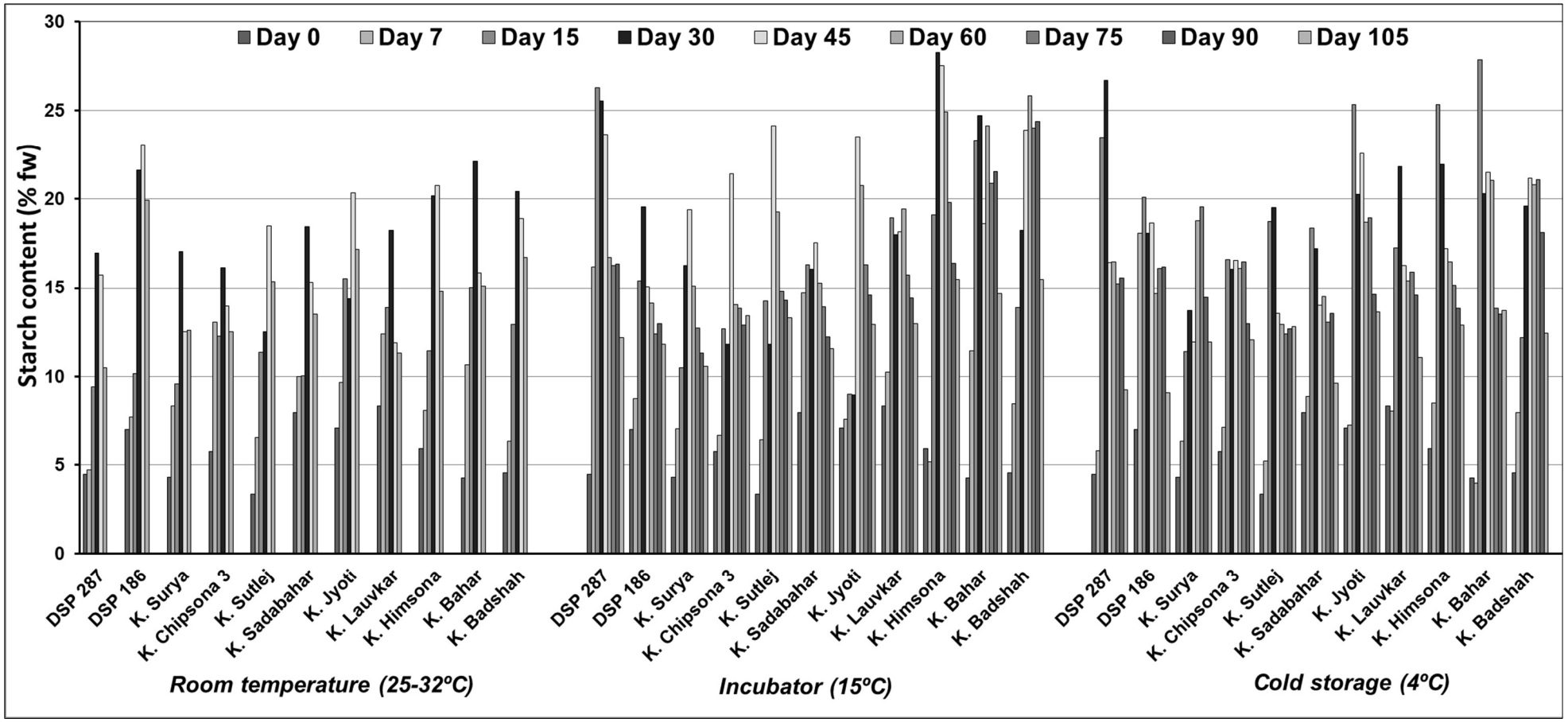

Figure 3. Variations of starch content of potato varieties stored at three different temperature. Soluble carbohydrates were washed from potato tubers powder, remaining starch was hydrolyzed with 6N HCl at 100°C and glucose released was estimated by phenol sulphuric acid method. The value of glucose content was multiplied by a factor 0.9 to obtain starch content.

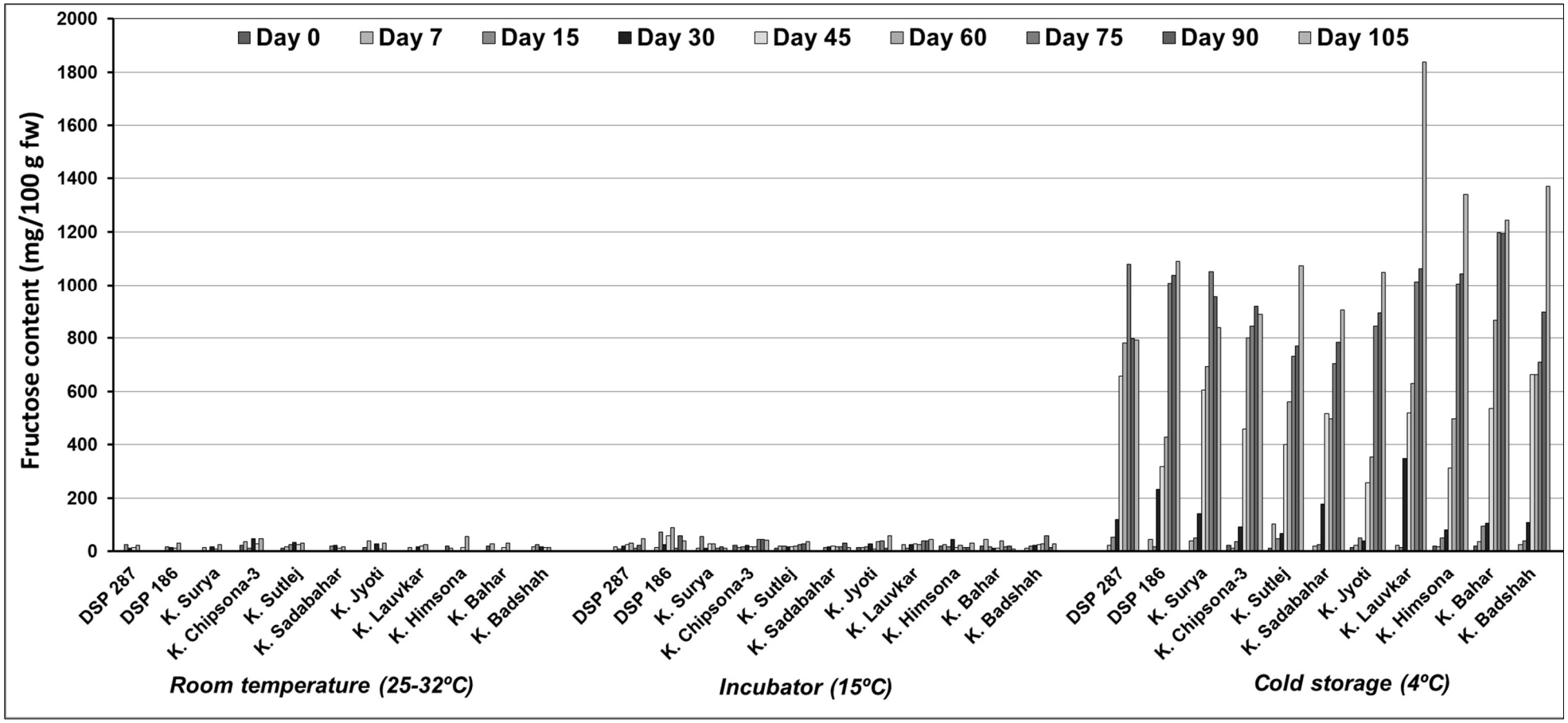

Figure 4. Changes in fructose content of potato varieties stored at three different temperature. Sugars were extracted in 80% methanol, filtered through a PVDF membrane and 20µL were separated through an Amino (NH_2) HPLC column at 1.8 mL/min flow rate of acetonitrile:water (80:20) mobile phase. Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times of standards and fructose content was calculated according to peak area.

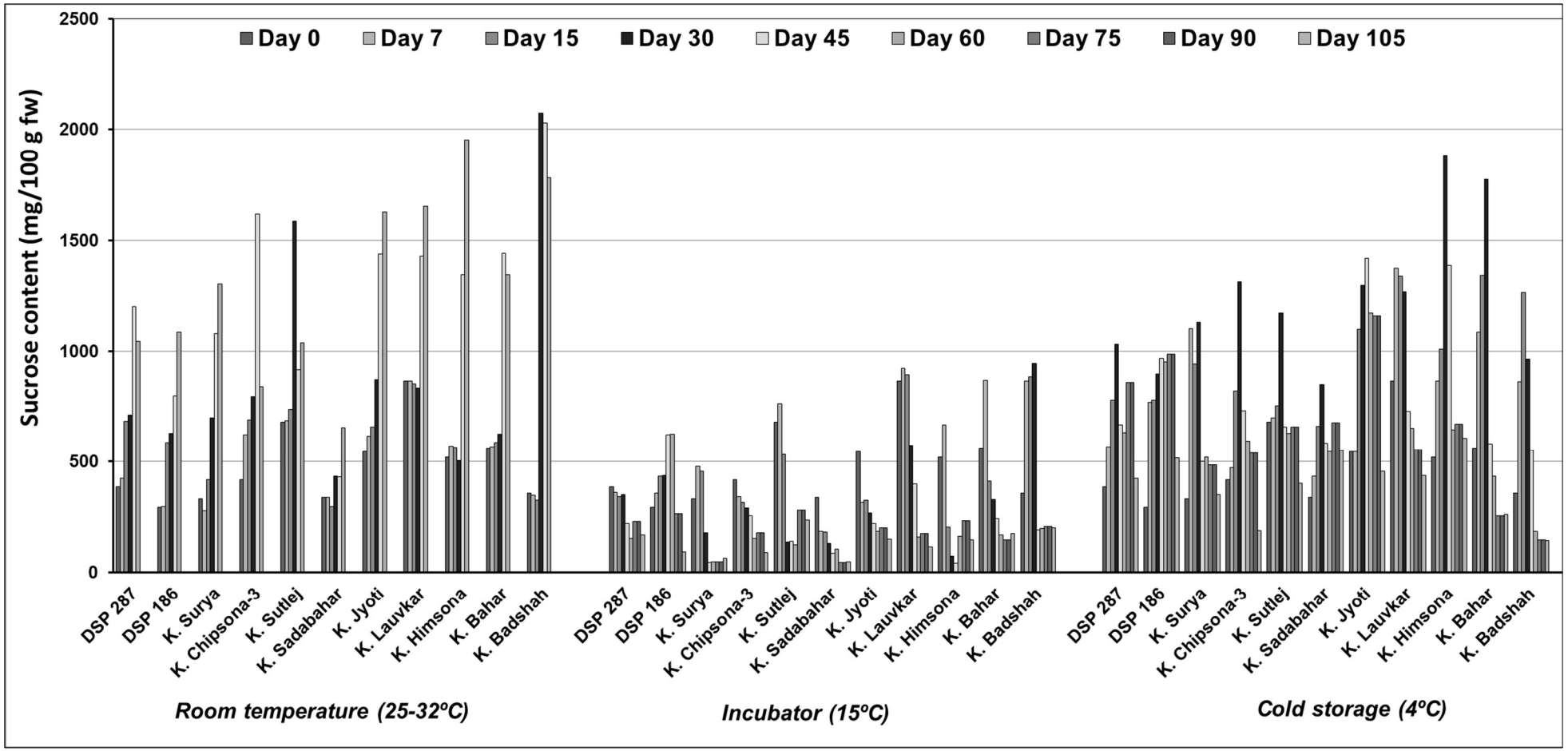

Figure 5. Changes in sucrose content of potato varieties stored at three different temperature. Sugars were extracted in 80% methanol, filtered through a PVDF membrane and 20µL were separated through an Amino (NH_2) HPLC column at 1.8 mL/min flow rate of acetonitrile:water (80:20) mobile phase. Chromatograms were integrated using Empower 3 software, peaks were identified by comparing with retention times of standards and sucrose content was calculated according to peak area.

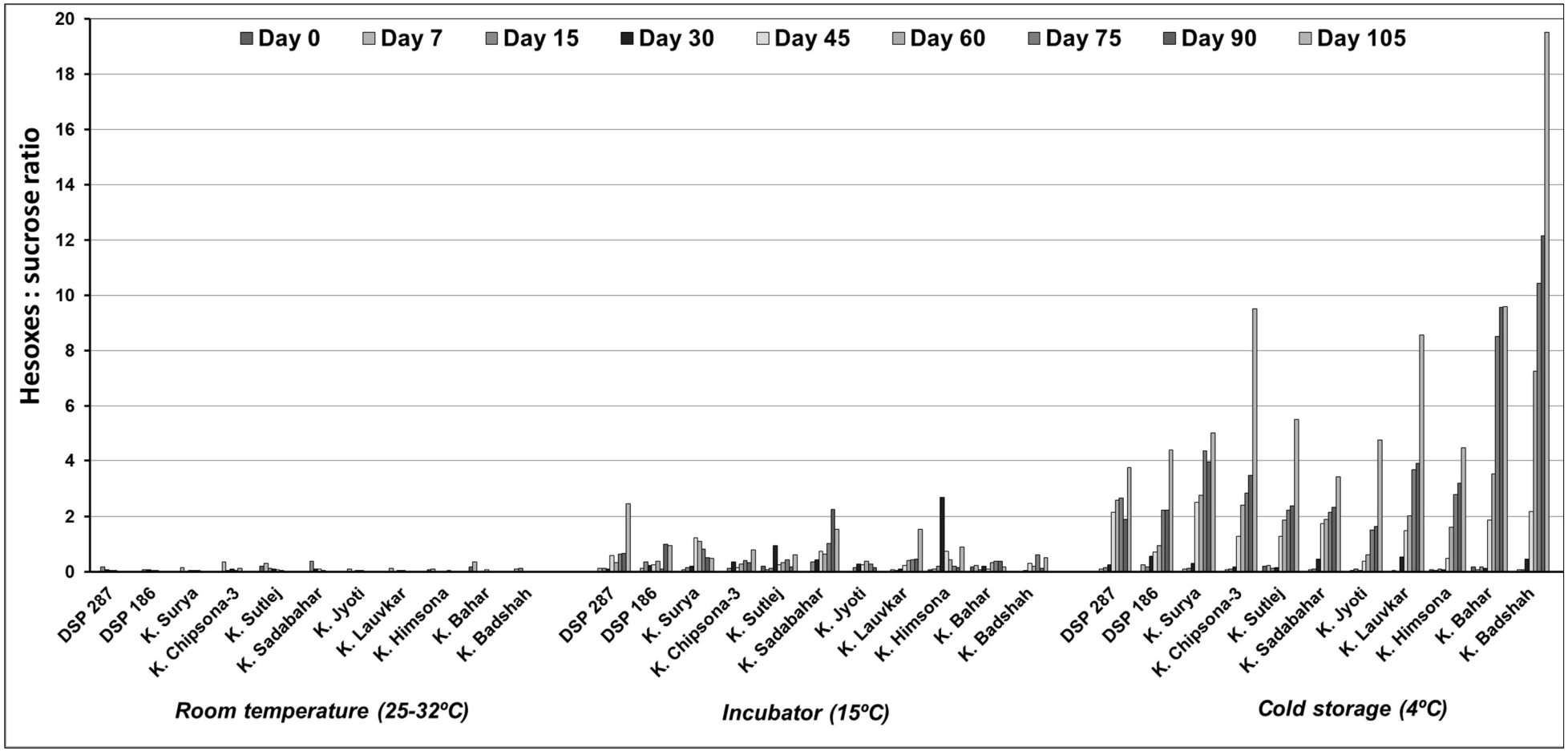

Figure 6. Difference in hexoses:sucrose ratio of potato varieties stored at three different temperatures. Sugars content was determined by HPLC and the ratio was calculated as sum of Glucose content + Fructose content divided by Sucrose content.

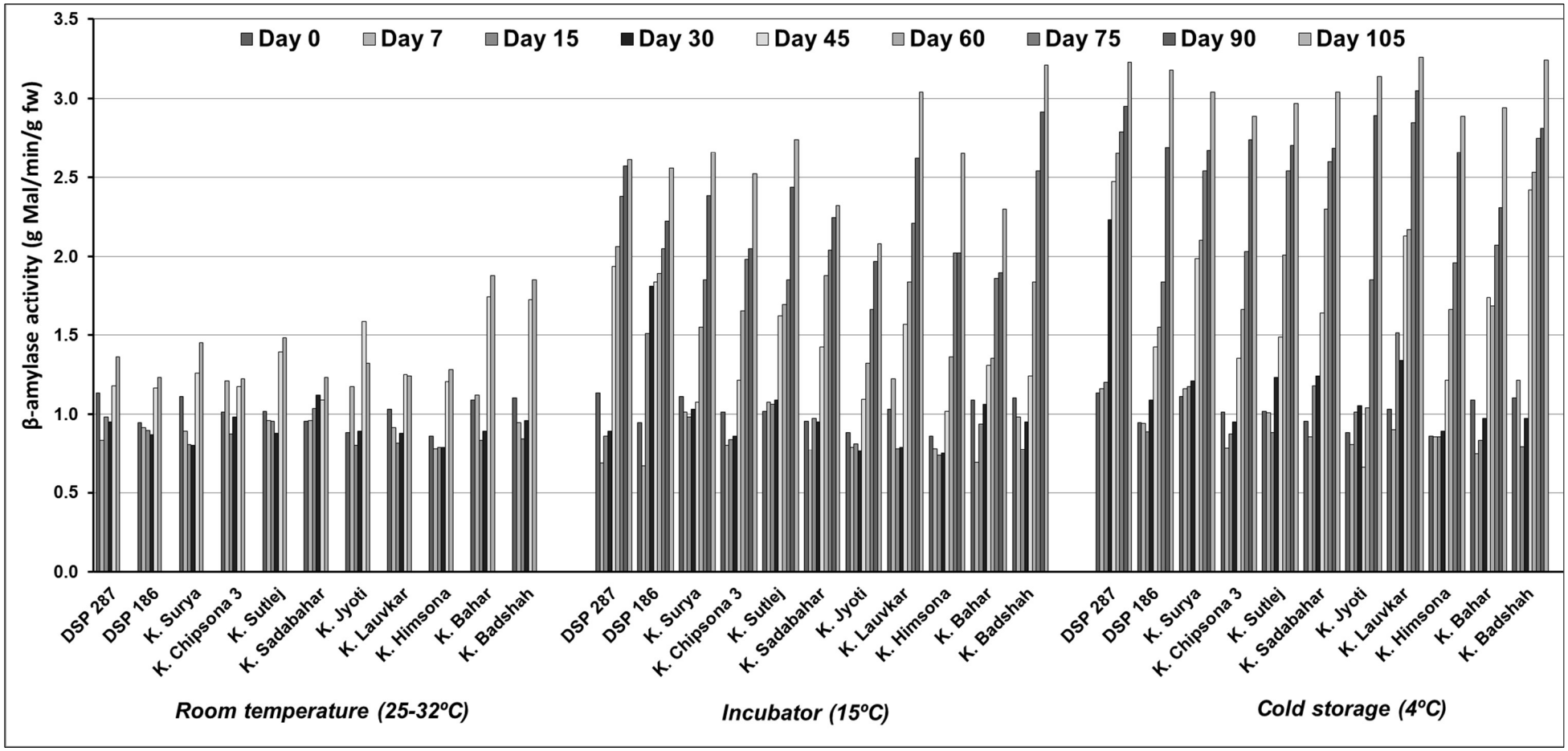

Figure 7. Changes in β-amylase activity of potato varieties stored at three different temperatures. Enzymes were extracted in Tris-acetate buffer, potato starch was used as substrate and activity of β-amylase was measured as maltose formed after 15 min of reaction.

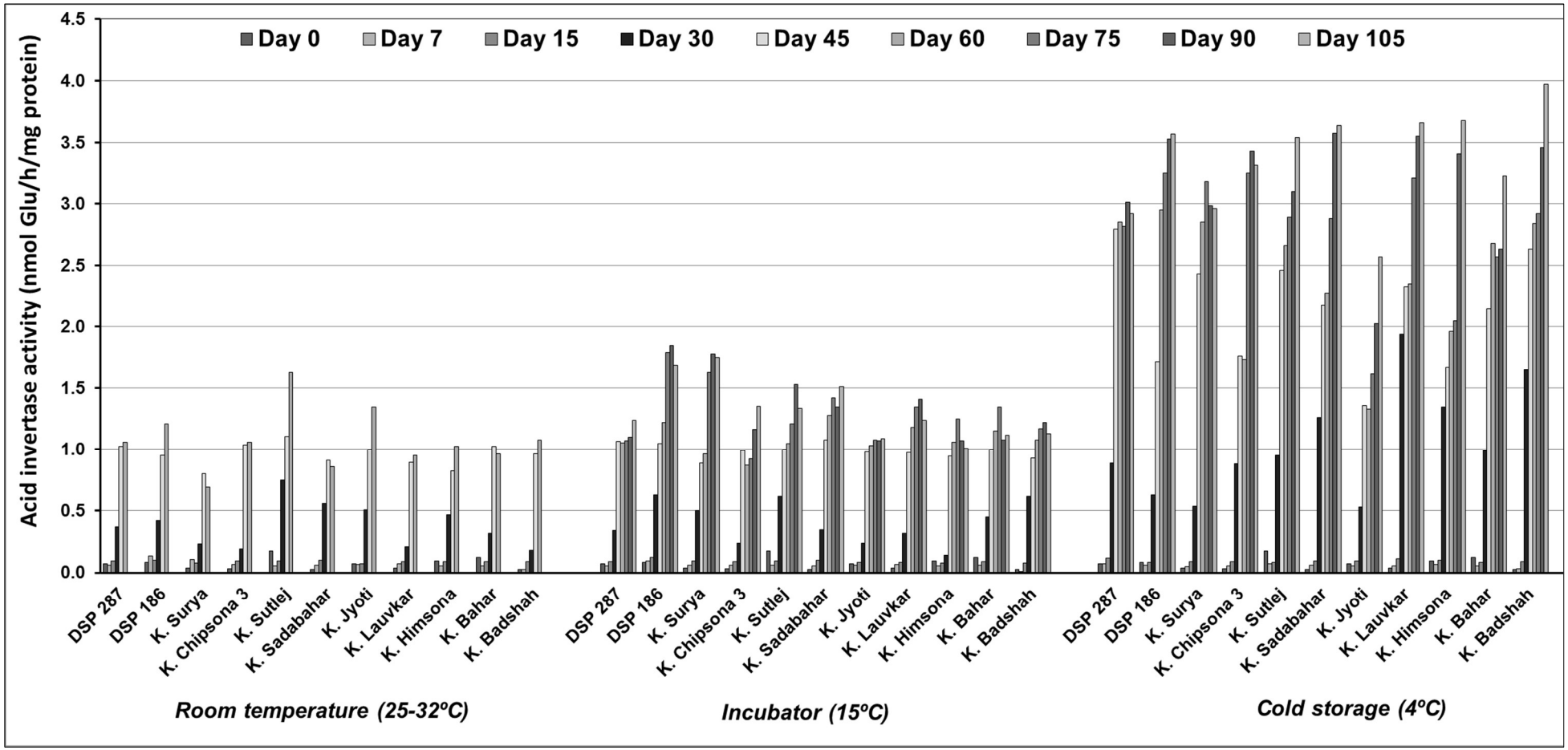

Figure 8. Changes in acid invertase activity of potato varieties stored at three different temperatures. Enzymes were extracted in HEPES-KOH buffer, sucrose was used as substrate and acid invertase activity was measured as glucose formed after 30 min of reaction.