JEB
Website : www.jeb.co.in
E-mail : editor@jeb.co.in

Journal of Environmental Biology

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

# Role of invertase activity in processing quality of potatoes: Effect of storage temperature and duration

**Bandana[1]*, Vineet Sharma[1], Brajesh Singh[2], Pinky Raigond[2] and S.K. Kaushik[1]**

[1]Crop Physiology, Biochemistry and Post Harvest Technology, CPRIC, Modipuram, Meerut-250 110, India
[2]Crop Physiology, Biochemistry and Post Harvest Technology, CPRI, Shimla-171 001, India
*Corresponding Author E-mail: **bandana.cpri@yahoo.com**

**Publication Info**

*Paper received:*
31 January 2015

*Revised received:*
11 July 2015

*Re-revised received:*
30 September 2015

*Accepted:*
31 October 2015

**Abstract**

Invertase activity and processing attributes of three potato cultivars were studied to find the reason for deterioration of processing quality during their prolonged storage in commercial cold stores (4°C) as compared to elevated temperature storage (12±0.5°C), with CIPC {Isopropyl-N-(3-Cholorophenyl) carbamate}. Lower storage temperature (4ºC) tended to be more effective in increasing invertase activity of potato tubers than elevated temperature. Non-processing cultivar *viz*., Kufri Pukhraj resulted in accumulation of more invertase activity than relatively two processing cultivars. Kufri Chipsona-1 and Kufri Chipsona-3 at 12±0.5°C possessed basal invertase activity ranging from 39.3 to 79.8 and 54.1 to 93.8 (µmoles hexose $h^{-1}$ $g^{-1}$ f.wt.) respectively, during two years. Total invertase activity at 4°C increased abruptly and remained high from 30 to 60 days of storage. The activity progressively reached 90.6 to 106.6 and 81.4 to 101.3 during both the years respectively, after 60 days of storage to that observed initially. Reducing sugar content increased from 23.3 to 105.7 and 389.0 to 1138.2 (mg $100g^{-1}$ f.wt.) after 90 days of storage at 12±0.5°C and 4°C, respectively. Studies concluded that basal and total invertase, were responsible for cold-induced sweetening and resulted in deterioration of processing quality of potatoes during storage at 4°C. Since this activity is low at 12±0.5°C, the processing traits remained acceptable to industry and consumers.

**Key words**

Chip colour, CIPC, Invertase activity, Reducing sugars, Storage temperature

## Introduction

Potato (*Solanum tuberosum* L.) is considered as one of the most important food throughout the world and has potential to provide food and nutritional security to population, especially in the developing world. Potato crop is seasonal, semi-perishable and hence cannot be stored for indefinite period in natural environment. Fresh potatoes thus need to be stored in cold storages to maintain round the year supply to the market and processing industry. Storage at low temperature (<8°C) is beneficial for long term storage as it reduces the bacterial soft rots, decreases dry matter loss and prevents sprouting without application of any sprout inhibitors (Sowokinos, 2001). However, cold storage of potato (at 4°C) triggers accumulation of sugars. In many cultivars, low temperature storage, leads to glucose and fructose to accumulate at substantial levels (Blenkinsop *et al*., 2004; Bradford *et al*., 2014). Since the respiration rate and sprouting are inhibited by low temperature, consumption of reducing sugars is low and their content accumulates in the tubers, resulting in dark colored chips. Sugars tend to accumulate when their production is more as compared to utilization. Besides storage temperature, physiological age of the tuber also affects sugar accumulation. Storage temperature lower than 6ºC increases the activity of enzymes that convert starch into fructose and glucose (Matsuura *et al.*, 2004). Potato storage at low temperature results in cold-induced sweetening that occurs due to imbalance between the rate of breakdown of starch and metabolism of the resulting sucrose, some of which enters the vacuole where it can be irreversibly cleaved by acid invertase to hexose sugars *i.e.,* glucose and fructose (Sowokinos, 2001; Blenkinsop *et al*.,

2004). The pathway from starch breakdown to hexose sugars is complex (Malone *et al*., 2006) and several enzymes may be potentially involved. For desired processing quality of potato tubers low reducing sugar are required (Abong *et al*., 2009) and for their storage, temperature above 10°C is desirable (Mehta and Ezekiel, 2006) but at this temperature sprouting needs to be controlled. Therefore, potatoes are stored at elevated temperature (12±0.5°C) with CIPC treatment (Isopropyl N-(3-Chlorophenyl) Carbamate) for effective control of sprouting. Most of the studies have been carried out at low temperature such as 2-4 ºC, this temperature though is suitable for storage of seed potatoes and it is not at all suitable for storing the potatoes meant for table and processing due to excessive accumulation of reducing sugars (Singh and Ezekiel, 2010). Therefore, potatoes for processing purpose are stored at 12±0.5°C with CIPC treatment. Storage and their biochemical behaviour and processing quality has not been carried out under commercial conditions in sub-tropical climate. Therefore, study aimed at finding out the changes in processing quality due to enzymatic activity of invertases at 12±0.5°C (elevated temperature storages) in comparison to 4 °C (common practice of cold storage in commercial cold storages for seed) during prolonged storage of potatoes.

## Materials and Methods

Three potato varieties *viz.* Kufri Chipsona-1, Kufri Chipsona-3 and Kufri Pukhraj were grown at Central Potato Research Institute Campus, Modipuram, Meerut during 2011-12 and 2012-13 following all the recommended cultural practices (Kumar *et al*., 2007). The crop was planted consecutively for two years during October and harvested after 120 days except for Kufri Pukhraj which was harvested at 90 days. Samples of 5kg each of three cultivars were stored in three replicates at 4°C and at 12±0.5°C for six months (after harvest to 180 days). Potato stored at 12±0.5°C were treated twice (first week of April and May) with 35ml tones$^{-1}$ (50% active ingredient) of CIPC {Isopropyl-N-(3-Chlorophenyl) carbamate}. Stored potatoes were sampled every month for invertase activity, chip colour and reducing sugar content i.e. at 0, 30, 60, 90, 120, 150 and 180 days after storage. Statistical analyses of experimental data were performed using statistical software 'IRRISTAT'developed by the International Rice Research Institute (IRRISTAT, 2005) using completely randomized block design.

Invertase activity was measured by the method suggested by Uppal and Verma (1990) with slight modification. Chopped samples (10g) were ground in pre-cooled 0.2M sodium acetate buffer (pH 4.75) using liquid nitrogen. Slurry was filtered through layers of muslin cloth and volume was made up to 50 ml. Extract was centrifuged at 5000g for 15 min and 25 ml of each extract was dialysed against extracting buffer for 26 hrs. For estimation of total invertase, 20ml of extracted sample was diluted with 0.2M sodium acetate buffer and stirred continuously at 37°C to inactivate endogenous inhibitor. Dialysed extract was used for estimation of basal invertase activity and dialysed and blended samples were used for analysis of total invertase activity. Colour was developed using arsenomolybdate solution and absorbance was measured at 620nm.

Reducing sugars in potato tubers were extracted by refluxing samples in 80% ethanol and analysed by the method of Nelson (1944) through colorimetric assay. Color developed by arsenomolybdate reagent was measured at 620 nm and concentration was calculated by preparing standard curve of glucose solution (100 µg ml$^{-1}$).

Potatoes were sliced (>1.75mm) washed in cold water, dried and fried in refined oil at 180°C till bubbling stopped. Chip colour was subjectively assessed by visual scoring of the colour on the scale of 1 to 10, where 1 denoted highly acceptable and scored up to 3 was considered acceptable (Ezekiel *et al.,*2003).

## Results and Discussion

Immediately after harvest, low basal invertase activity was observed in all the cultivars. During 2012 storage, both basal, as well as, total invertase increased up to 90 days of storage and declined at 120 days (Fig 1A). Whereas, in 2013 season, the basal and total invertase activity increased up to 120 days of storage and decreased thereafter (Fig 1B). Processing cultivars viz., Kufri Chipsona-1 and Kufri Chipsona-3 had low values of both activities as compared to Kufri Pukhraj at 12±0.5°C. During first and second year of experiment, values for basal invertase activity ranged from 39.3 to 79.8 and 54.1 to 93.8 (µmoles hexose h$^{-1}$ g$^{-1}$ f.wt.) respectively, at 12±0.5°C. Total activity was more than basal activity at all the sampling stages and ranged from 55.1 to 95.8 (µmoles hexose h$^{-1}$ g$^{-1}$ f.wt.) during first year and 68.1 to 107.9 (µmoles hexose h$^{-1}$ g$^{-1}$ f.wt.) during second year, respectively. Initially, total invertase activity increased 0.7-0.9 fold at 12±0.5°C, whereas 0.8-1.0 fold was noticed at 4 °C. Storage of potato tubers at low temperature (4°C) accumulated higher concentration of basal and total invertase activity than tubers stored at elevated temperature. The results obtained is in agreement with the previous results at low temperature storage of tubers, suggesting rapid conversion of starch to hexose along with increase in invertase (Zommick *et al*., 2014). Increase in enzyme activity at 4°C was highest (108.2 µmoles hexose h$^{-1}$ g$^{-1}$ f.wt.) in Kufri Pukhraj followed by Kufri Chipsona-3 (81.3 µmoles hexose h$^{-1}$ g$^{-1}$ f.wt.) at 120 days of storage. During both the years of experiments, changes in invertase activity were similar and affected accumulation of reducing sugars (Fig. 3A, B), and

differences were apparent between processing and non-processing varieties. Invertase enzyme in tubers is a key enzyme which controls the reducing sugars. The basal invertase activity in tubers was low which might be due to excess of invertase inhibitor at fresh harvest. Storage of tubers at low temperature might have resulted in rapid conversion of starch to sugars due to enzymatic activity, and after sufficient duration of storage at a given temperature maximum sugar was attained and total invertase activity start declining. Low sugar and high sugar cultivars in the present study indicated a strong correlation between glucose content and acid invertase basal activity in tubers stored for two months at 7°C ($r = 0.77$); correlation was found to be stronger when data of different storage stages were performed ($r = 0.87$), suggesting that basal activity of acid invertase might play a major role in determining sugar content in cold stored tubers (Xu *et al*., 2009). The effect of invertase activity on reducing sugar accumulation and the processing quality of potato tubers (4°C) had significant linear correlation suggesting that acid invertase could play a major role in the cold induced sweetening of potato tubers through regulation of starch-sugar metabolism (Cheng et al., 2004).

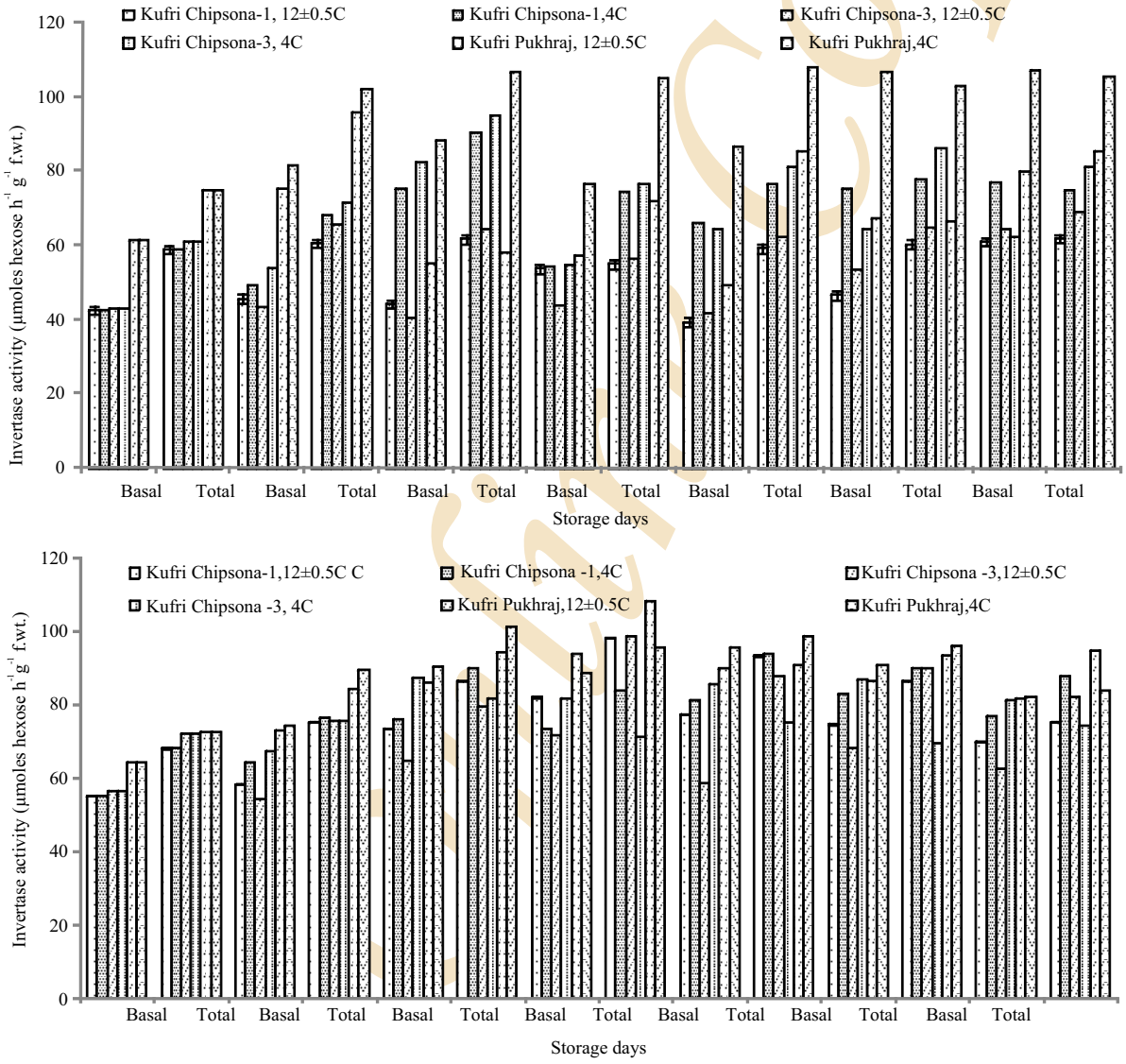


**Fig. 1 A, B :** Invertase activity as influenced by storage temperature and duration A (2011-12) and B (2012-13). Error bars represent standard error values of 5% level of significance

Potato chip colour was highly acceptable immediately after harvest of the crop in cv. Kufri Chipsona-3 (Fig. 2A) during first year of experiment (2011-12). It was observed that potatoes stored at 4°C had high chips color score (dark brown colour) in comparison to potatoes stored at 12±0.5°C. Similar results were observed previously (Kumar *et al*., 2004) and it was reported that low temperature stored potatoes on processing into chips lead to reaction between reducing sugars and free amino acids by Maillard reaction and resulted in an unacceptable browning of the product. Among the three varieties, processing of cv. Kufri Chipsona-3 maintained consistency in acceptable colour range (3.0) up to 150 days of storage (12±0.5°C) in comparison to other two varieties under test *viz.* Kufri Chipsona-1 and Kufri Pukhraj. However, potato chip of colour score at 4°C were unacceptable in all the cultures. The acceptable colour of potato chips in processing cultivars at 12±0.5°C could be due the fact that starch might not have been converted to reducing sugar at elevated temperature. During second year of experiment (2012-13), Kufri Chipsona-1 retained acceptable potato chip colour up to 120 days of storage (Fig. 2B). Whereas, variety Kufri Chipsona-3 showed acceptable chip colour at all sampling dates except at 90 days of storage. In contrast, the potato chips prepared from variety Kufri Pukhraj were not acceptable at any of the sampling dates during both the years. This could be due to initial high level of sugar or storing potato at low temperature influencing accumulation of glucose and fructose that results in dark

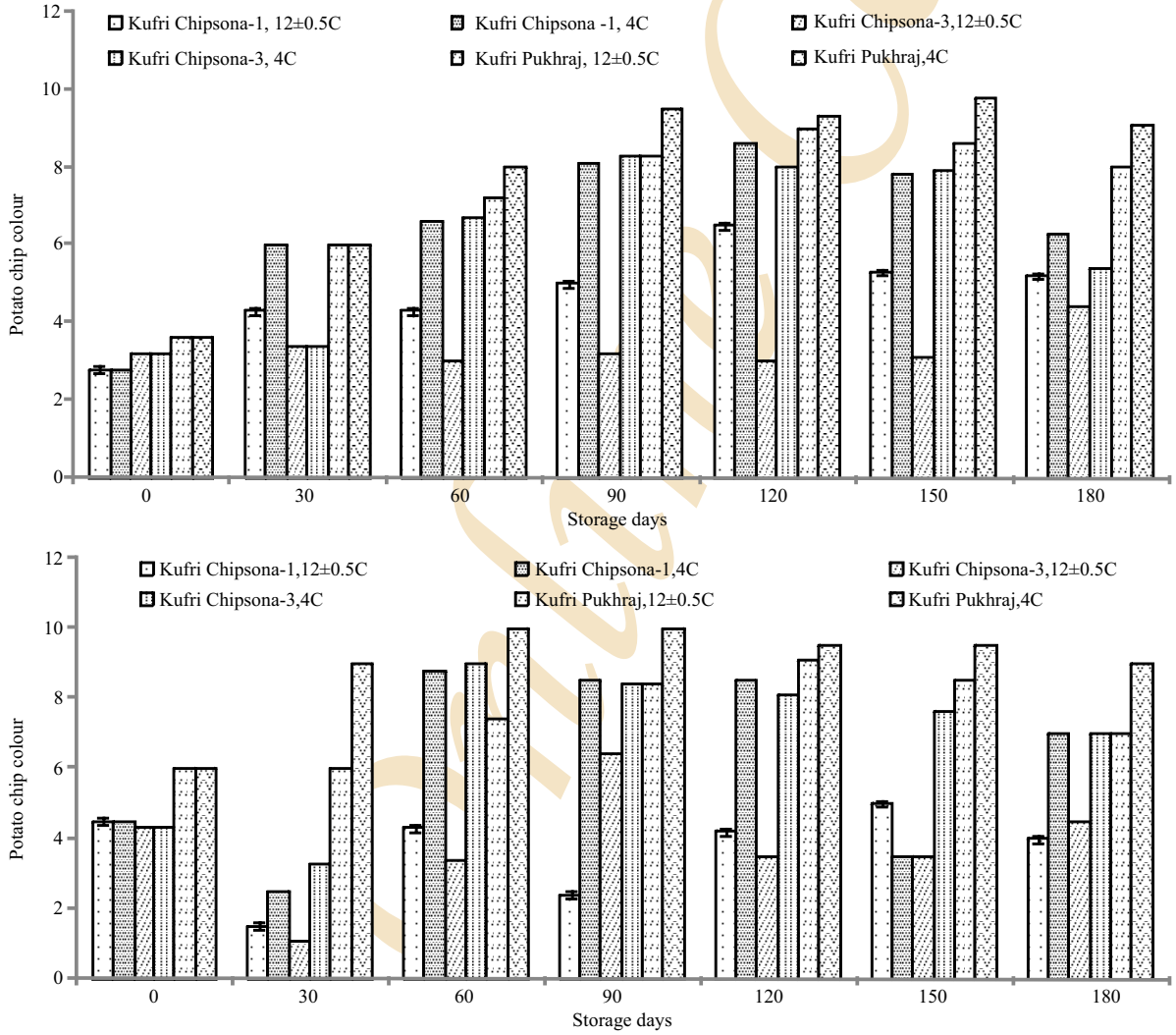


**Fig. 2 A, B :** Potato chip colour as influenced by storage temperature and duration A (2011-12) and B (2012-13). Error bars represent standard error values of 5% level of significance

coloring of potato chips during frying process of the potatoes (Knowles *et al*., 2009). During low temperature, sugars generally accumulate as a way to regulate the osmotic potential and as cryo-protectant. Moreover, sucrose due to activation of invertase enzyme,breakdown into glucose and fructose leading to sweetening of potatoes (Xu *et al*., 2009). Invertase in potato tuber is a key enzyme, that control reducing sugars and seems to affect enzymatic and non-enzymatic reactions responsible for changes in texture, colour and taste of the product during storage (Zare *et al.,* 2002).

Storage of potato tubers at low temperature (4 ºC) lead to significant accumulation of reducing sugars (mg 100 $g^{-1}$ f.wt.) in all the cultivars and accumulation was more at lower temperature (4 ºC ) as compared to elevated temperature with CIPC (12±0.5ºC). The effect of storage period was found to be significant during the both the years of experiment. Different varieties behaved differently when stored at different temperatures. Reducing sugar content of variety Kufri Chipsona-1 increased from 30 to 90 days of storage and declined at 120 days during first year of experiment at 12±0.5°C. Whereas during second year of experiment,

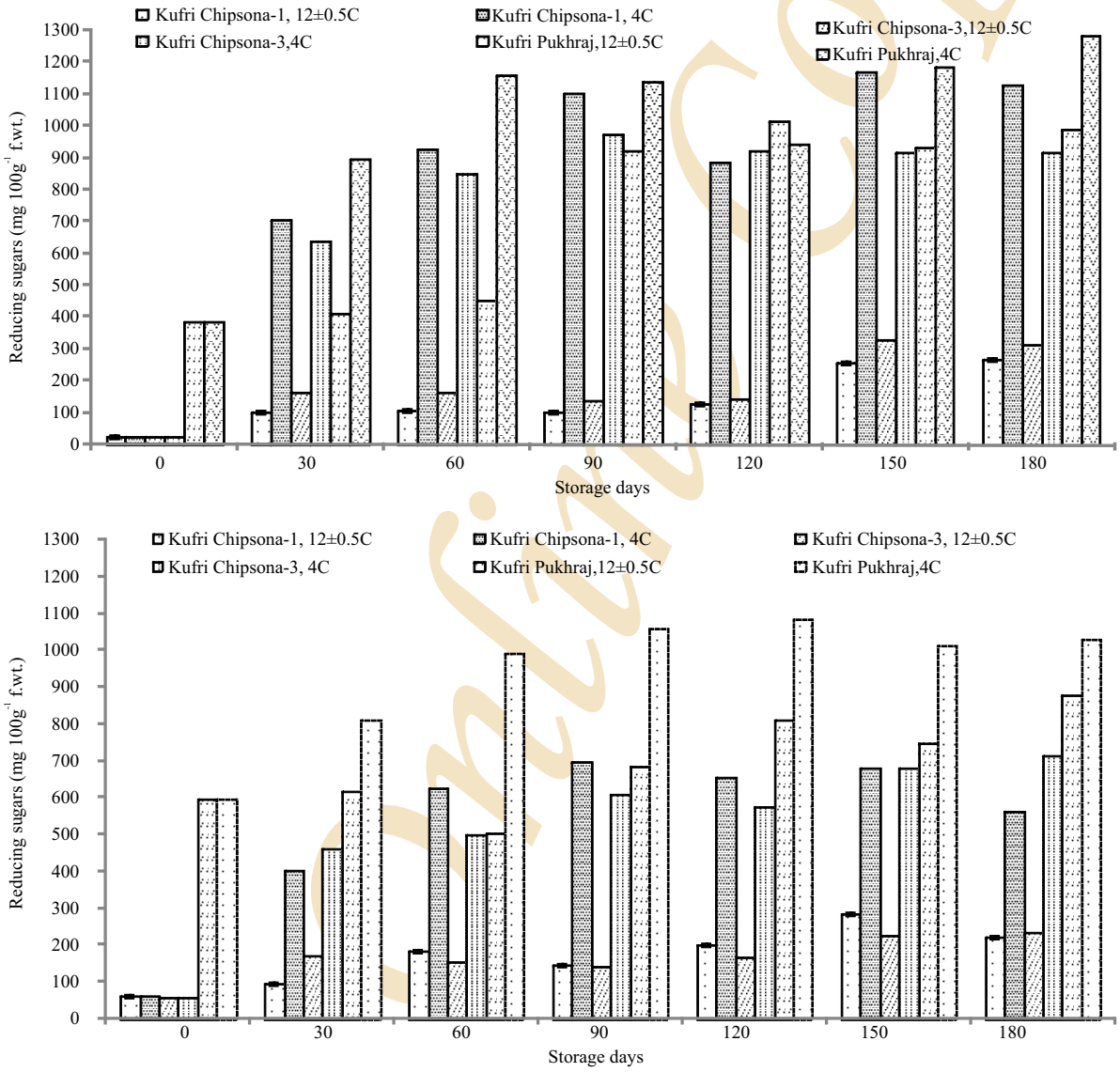


**Fig. 3A, B:** Reducing sugars as influenced by storage temperature and duration A (2011-12) and B (2012-13). Error bars represent standard error values of 5% level of significance

content increased from 30 to 150 days of storage except at 90 days. Reducing sugar content was found acceptable (<100 mg $100g^{-1}$ f.wt.) in processing cultivars *viz*., Kufri Chipsona-1 and Kufri Chipsona-3 at 0 (23.3 mg $100g^{-1}$ f.wt. in first year and 61.4 mg $100g^{-1}$ f.wt. in second year) and 30 days (98.8 mg $100g^{-1}$ f.wt. in first year and 95.8 mg $100g^{-1}$ f.wt. in second year), respectively. Sugar content of cv. Kufri Chipsona-3 increased at 30 days (Fig.3A), decreased from 60 to 120 days and again increased at 150 days of storage at 12±0.5°C (2011-12). Whereas, same variety indicated different trend during second year of experiment where, reducing sugar content increased from 30 to 180 days of storage (except at 60 and 90 days of storage). Non- processing cultivar *i.e.,* Kufri Pukhraj accumulated more reducing sugar (irrespective of both the temperatures) as compared to processing varieties *viz*., Kufri Chipsona-1 and Kufri Chipsona-3 (Fig. 3B). All the varieties except for Kufri Pukhraj had low reducing sugars when sampled at zero days, which is in accordance with previous findings (Ezekiel *et al*., 2007). Similar trend was also observed during second year of experiment, where sugar content ranged from 59.4 to 167.4 mg $100g^{-1}$ f.wt. in variety Kufri Chipsona-3 followed by another processing variety Kufri Chipsona-1 (61.4 to 203.1 mg $100g^{-1}$ f.wt.). Significant varietal differences were observed on the effects of storage temperature on concentration of reducing sugars and colour of chips during both the years. Increase in reducing sugar content at low temperature has also been reported (Kevin *et al*., 2000; Murata *et al*., 2000). According to one storage study (Singh *et al*., 2008) majority of cultivars could increase hexose content, ultimately affecting quality of end product. Carbohydrate content of potatoes also depends on variety and physiological state of the tubers, carbohydrates content may change during tuber development and during the time of storage (Hofius and Bornke, 2007). At low temperature some of the carbohydrate splitting enzymes may get activated and play important role for the sweetening of potatoes (Karim *et al.,* 2008). Reducing sugar levels were significantly higher at low temperature than potatoes stored at 12±0.5°C (CIPC). During cold storage of tubers, starch may have remobilized and converted to sugars. Starch may be degraded either hydrolytically or phosphorolytically, and products exported from amyloplasts either as hexose phosphates (hexose-P) *via* glucose phosphate–phosphate translocator or as free sugars via glucose. Correlation studies for invertase activity versus reducing sugars was worked out during both the years. During first and second year of experiment, a positive correlation between invertase and reducing sugars was observed from beginning (0.99 and 0.58) till end of storage season (0.82 and 0.75), except at 90 days of sampling (during second year) where correlation was found to be negative (-0.68). Likewise, invertase activity had positive correlation with potato chip colour at all the sampling stages except at 90 days of storage. Potato chips colour indicated positive correlation with reducing sugars at all the stages of sampling. Invertase activity influenced the processing quality of potatoes by conversion of starch to sugars and activity being higher at low temperature. The resultant increase in sugars ultimately led to deterioration in processing quality of tubers.

It may be concluded that temperature had profound effect on invertase activity resulting in increased reducing sugar level in potato tubers, ultimately affecting colour of potato chips. Invertase has been found to play an important role in hydrolysis of sucrose to glucose and fructose, especially in the storage organs, although other factors may be involved in regulation of reducing sugar levels of potato.

## Acknowledgment

Authors are thankful to the Director, Dr. BP Singh, CPRI, Shimla for providing the facilities and financial assistance in terms of institute project.

## References

Abong, G.O., M.W. Okoth E.G. Karuri, J.N. Kabira and F.M. Mathooko: Evaluation of selected Kenyan potato cultivars for processing into French fries. *J. Ani. P. Sci.,* **2**, 141-147 (2009).

Blenkinsop, R.W., R.Y. Yada and A.G. Marangoni: Metabolic control of low-temperature sweetening in potato tubers during post-harvest storage. *Hort. Rev*., **30**, 317–354 (2004).

Bradford, A., E.W. J.S. Busse, J. Jiming and P.C. Bethke: Sugar metabolism, chip color, invertase activity and gene expression during long-term cold storage of potato (*Solanum tuberosum* L.) tubers from wild-type and vacuolar invertase silencing lines of Katahdin. *BMC Res. Notes.*, **7,** 801-810 (2014).

Cheng, S., Z.H. Su, C.H. Xie and J. Liu: Effects of variation in activities of starch-sugar metabolic enzymes on reducing sugar accumulation and processing quality of potato tubers. *Agric. Sci. China.,* **7**, 519-527 (2004).

Ezekiel, R., B. Singh and D. Kumar: A reference chart for potato chip colour for use in India. *J. Ind. Potato Assoc.,* **30,** 259-265 (2003).

Ezekiel, R., B. Singh, D. Kumar and A. Mehta: Processing quality of potato varieties grown at two locations and stored at 4, 10 and 12°C. *Potato J*. **34**, 164-173 (2007).

Hofius, D and F.A.J. Bornke: Photosynthesis, carbohydrate metabolism and source-sink relatios. *In:* Potato biology and biotechnology. Adv. and perspective, (Eds. Vreugdenhil, D., J. Bradshaw, C. Gebhardt, F. Govers, D.K.L. MacKerron, M. A. Taylor and H. A. Ros ) pp. 257-285. (2007).

IRRISTAT software 5.0 for windows. International rice research Institute, Manila. Phillipines (2005).

Karim, M.D.R., M.M.H. Khan, M.D.S. Uddin, N.K. Sana, F. Nikkon and M.D.H. Rahman: Studies on the sugar accumulation and carbohydrate splitting enzyme levels in post harvested and cold stored potatoes. *J. Bio.Sci.*, **16**, 95-99 (2008).

Kevin, F.Y., H. Sato, T. Suzuki, K. Takano and T. Kozima: Sugar accumulation as a mechanism for freezing tolerance in cold-stored 'Danshaku' potato tuber (*Solanum Tuberosum* L.). *Food Preser. Sci.,* **26**, 103-107 (2000).

Knowles, N.R., J.R.E.P.Driskill and L.O.Knowles: Sweetening

responses of potato tubers of different maturity to conventional and non-conventional storage temperature regimes. *Postharvest Biol.Tech.* **52**, 49-461 (2009).

Kumar, D., B.P. Singh and P. Kumar: Current status and new vistas in potato processing quality research. *In*: Processing and export potential of Indian potatoes (Ed: SMP Khurana *et al*., Eds.), Indian potato association, CPRI, Shimla, India pp. 55-60 (2004).

Kumar, P., S.K., Pandey, S.V. Singh and D. Kumar: Irrigation requirements of chipping potato cultivars under west-central Indian plains. *Potato J.,* **34***,* 193-198 (2007).

Malone, J.G., V. Mittova, R.G. Ratcliffe and N.J. Kruger: The response of carbohydrate metabolism in potato tubers to low temperature. *P. Cell Physiol*., **47**, 1309–1322 (2006).

Matsuura, E.C., A. Kobayashi, T. Noda, S. Takigawa, H. Yamauchi and M. Mori: Changes in sugar content and activity of vacuolar acid invertase during low-temperature storage of potato tubers from six Japanese cultivars. *J. Plant Res.,* **117**, 131–137 (2004).

Mehta, A. and R. Ezekiel: Storage of potatoes: Need, present scenario, emerging technologies and future strategies: A critical Review. *J. Food Sci. Technol*. **43**, 453-466 (2006).

Murata, Y.,T.K. Noguchi, F.Y. Sato, H. Matsumoto, S.T.K. Takano and T. Suzuki: Amylase and invertase activity and substrate affinity in 'Danshaku' potato tuber (*Solanum Tuberosum* L.) during cold storage. *Food Preser. Sci*., **26**, 281-287 (2000).

Nelson, N.: A photometric adaption of the Somogyi's method for determination of glucose. *J. Biol. Chem.,* **153**, 375-380 (1944).

Singh, B. and R. Ezekiel:Isopropyl n-(3-chlorophenyl) carbamate (cipc) residues in potatoes stored in commercial cold stores in india. *Potato Res.,* **53**, 111-120 (2010).

Singh, B., R. Ezekiel, D. Kumar and S. Kumar: Reducing sugars content and chipping quality of tubers of potato cultivars after storage and reconditioning. *Potato J*. **35,** 23-30 (2008).

Sowokinos, J.R.: Biochemical and molecular control of cold induced sweetening in potatoes. *Amer. J. Potato Res*., **78**, 221–236 (2001).

Uppal, D.S. and S.C.Verma: Changes in sugar content and invertase activity in tubers of some Indian potato varieties stored at low temperature. *Potato Res.,* **33**, 119-123 (1990)**.**

Xu, C., W.K.Coleman, F.R. Meng, M. Bonierbale and X.Q. Li: Relationship between glucose accumulation and activities of acid invertase and its inhibitors in potatoes under simulated commercial conditions. *Potato J.,* **36**, 35-44 (2009).

Zare, Z., M.Sohrabpour, T.Z. Fazeli and K.G Kohan: Evaluation of invertase (B-fructo furanosidase) activity in irradiated mazafaty dates during storage. *Radiation Phy. Chem.,* **65**, 289-291 (2002).

Zommick, D.H., L. Knowles and N. Knowles: Tuber respiratory profiles during low temperature sweetening (LTS) and reconditioning of LTS-resistant and susceptible potato cultivars. *Postharvest Bio. Tech.,* **92**,128–138 (2014).