Cold-induced sweetening development in Indian potato (Solanum tuberosum L.) varieties

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Received 28 June 2010; revised 23 December 2010

Developing cold resistant sweetening processing varieties is one of the front areas of research all over the world. In India, first potato processing variety was released in the year 1998 and till 2005 three varieties have been developed. But, there is no information available regarding sugar accumulation response of Indian varieties to low temperature storage. Therefore, it is imperative to generate basic information on cold sweetening development in Indian processing varieties for the use of potato breeders. Development of cold-induced sweetening and its relation to phenolic content of the tuber was studied in three Indian potato varieties viz., Kufri Chipsona-1, Kufri Chipsona-3 and Kufri Jyoti. The reducing sugars decreased in initial phase of storage, followed by continuous increase to unacceptably higher levels after around two weeks of storage. The increase in reducing sugar contents took place subsequent to increase in sucrose content. The changes in phenol content were not in a fixed trend. The degree or number of folds increase in reducing sugar content was relatively less in Kufri Jyoti which contained highest phenol content among the three varieties investigated. It is suggested that development of processing varieties with higher anti-oxidant content and lower invertase activity may provide better cold-induced sweetening resistance.

Keywords: Potato, Reducing sugars, Sucrose, Phenols, Cold induced sweetening

To make fried or dehydrated potato products, the reducing sugar content of potato tuber should be low (<100 mg/100 g fresh weight for chips; <200 mg/100 g fresh weight for French fries and flakes). The higher concentrations of these sugars lead to development of undesirable brown colour and bitter taste upon frying or dehydration at high temperatures. These off-colored and off-flavored compounds are products of ‘Maillard reaction’ between reducing sugars and several nitrogenous compounds. The higher reducing sugars content is also related to formation of acrylamide (a potential carcinogenic compound) in fried potato products.

The content of reducing sugars in potato tubers is decided principally by its genetic make up and is affected by environmental conditions. Majority of potato crop in India is harvested in February/March and then stored in cold storage to provide round the year supply to consumers. But, storage at low temperatures (<10°C) results in excessive accumulation of free sugars in a phenomenon known as ‘cold-induced sweetening’, making the potatoes unsuitable for processing into fried or dehydrated products. Cold-induced sweetening (CIS) or low temperature sweetening (LTS) is a stress phenomenon that occurs in various parts of higher plants exposed to lower than optimum growth or storage temperatures (i.e. 10°C) and results in accumulation of polysaccharide breakdown products.

Several mechanisms have been proposed for LTS development in potato, but still the picture is not clear. The genotypes differ in their ability to form glucose during storage at low temperatures. Most of the studies on CIS have been conducted in potatoes grown under temperate climates, whereas majority of the potato crop is grown under sub-tropical climates in India. The sub-tropical growth and post-harvest environmental conditions may result in different storage behavior of potatoes with respect to sugar accumulation. There is no information available on LTS development in Indian potato processing cultivars.

It has been hypothesized in one of the study that development of reactive oxygen species at low temperatures affects membrane permeability through lipid peroxidation, leading to development of LTS and, therefore, anti-oxidative potential of potato tubers should be considered for further cultivar development. Potato tuber contains variety of anti-oxidant compounds, among them phenolics are the major one (58 to 82%) contributing to the total anti-oxidant activity. Therefore, changes in total phenol content during low temperature storage have also been monitored in this study.

In this study, attempt has been made to generate preliminary information regarding sugar accumulation pattern in Indian processing varieties Kufri Chipsona-1,
Kufri Chipsona-3 and traditionally used variety Kufri Jyoti. In addition, the changes in total phenolics at low temperature storage and its possible relationship with reducing sugar accumulation have also been investigated.

Materials and Methods
The potatoes grown with recommended package and practices at Central Potato Research Institute Campus, Modipuram farms were used for this study. The healthy and well cured tubers of varieties Kufri Chipsona-1, Kufri Chipsona-3 and Kufri Jyoti weighing approximately 60-80 g were stored in an incubator set at 4.5 ± 0.5°C. At each sampling interval, three tubers were drawn per replication and there were total three replications. The tubers were immediately cut into two pieces longitudinally and half part was chopped into small pieces, which were thoroughly mixed to make a homogenous sample. Tuber pieces (10 g) were put into 50 ml of boiling 80% isopropyl alcohol and content was boiled for 2 min, cooled and capped for further analysis. The reducing sugars, sucrose and phenols were extracted through repeated refluxion at 70°C in 80% isopropyl alcohol.

The extracted reducing sugars were quantified by Nelson’s method, sucrose by van Handel’s method and total phenols by Folin-Ciocalteau reagent. All analyses were done in duplicate and replicated data were statistically analyzed for variance using statistical software IRRISTAT.

Results
The effect of low temperature storage on reducing sugars, sucrose and phenolic contents of tuber was as under:

Reducing sugars
The statistical analysis showed significant effects (at 5% level) of storage period, variety and interaction between period and variety. The mean reducing sugar content started decreasing right from 4 h of storage and a significant decrease of 66.9 mg/100 g fresh wt was observed after 60 h of storage (Fig. 1). Thereafter, the content started increasing consistently and reached to the levels of 453.7 mg/100 g fresh wt after 35 days of storage. Among the three varieties, the mean reducing sugar content was maximum in Kufri Jyoti (181.8 mg/100 g fresh wt), while the difference between Kufri Chipsona-1 and Kufri Chipsona-3 (Fig. 1) was non-significant (contents 98.6 and 88.0 mg/100 g fresh wt, respectively). When individual varieties were compared at different storage periods, a significant decrease in reducing sugars was observed, even after 4 h of low temperature exposure in case of Kufri Jyoti. The content in other two varieties also started decreasing from this stage, but it was statistically non-significant. The content reached the undetectable levels in Kufri Chipsona-1 and Kufri Chipsona-3 after 72 h of storage, while at this stage reducing sugars started increasing in Kufri Jyoti. An upward trend in sugars of Kufri Chipsona-1 and Kufri Chipsona-3 started from 96 h onward. The reducing sugar content increased to significantly higher levels as compared to 0 h after 17 days of low temperature storage. At subsequent periods, the sugar accumulation continued to significant levels in Kufri Chipsona-1 and Kufri Chipsona-3.

Sucrose
The effect of storage period was significant (at 5% level), while for variety and interaction between variety and period, it was non-significant. The non-
significant interaction effect implied uniform effect of storage conditions on all the three varieties. A significant increase in mean sucrose content was observed after 72 h of storage (Fig. 2). At subsequent intervals, the increase continued till 7 days, remained statistically unchanged up to 17 days and then reached at peak at 21 days. The content decreased again at 28 days and remained statistically at par at 35 days of storage (Fig. 2).

**Phenols**

The effect of storage period, variety and interaction between the two was significant (at 5% level) for phenolics. The highest mean phenol content was observed in Kufri Jyoti (30.7 mg/100 g fresh wt), followed by Kufri Chipsona-3 (17.4 mg/100 g fresh wt) and lowest in Kufri Chipsona-1 (15.7 mg/100 g fresh wt). Consequent to storage at low temperature, the total phenol content decreased up to 8 h then increased at 12 h, followed by a zigzag pattern till 35 days (Fig. 3). When varietal behavior at different storage intervals was studied, the highest initial content (at 0 h) was in Kufri Jyoti (31.2 mg/100 g fresh wt), while the difference between Kufri Chipsona-1 and Kufri Chipsona-3 was non-significant (Fig. 3). After 8 h of storage, phenolics decreased to significant levels in all the three varieties. Then at 12 h the content started increasing with a significant increase only in Kufri Jyoti. The levels comparable to 0 h level were obtained in Kufri Chipsona-3 after 36 h and in Kufri Chipsona-1 after 48 h. At 96 h of storage, the phenol content was more than 0 h levels in Kufri Chipsona-1 and Kufri Jyoti. After 7 days of storage, the content again decreased in Kufri Chipsona-1, remained static in Kufri Chipsona-3 and Kufri Jyoti, as compared to 96 h. At 10 days stage, the content decreased in Kufri Chipsona-1 and Kufri Jyoti, while remained statistically at par in Kufri Chipsona-3. The changes after 10 days were statistically non-significant, as compared to 10 days.

**Discussion**

This is the first report describing the cold-induced/low temperature sweetening development in...
Indian potato processing varieties on a time scale. Surprisingly, the reducing sugars decreased during the initial period of storage before increasing to the unacceptably higher levels. The initial decrease could be because of spurt in respiration or popularly called respiratory burst upon shifting the tubers to lower temperatures. The higher rate of respiration might have consumed the reducing sugars. Respiration decreases as storage temperature decreases, but at storage temperature below 5°C, respiration is stimulated. There is a brief respiratory burst, followed by a subsequent decrease in respiration rate to a new steady state. The initial respiratory burst has been attributed to the combination of cytochrome-mediated and cyanide resistant respiration.

Workman et al have reported an increase in tuber respiration within 24 h at 0°C and the increase preceded the increase in sugars. They postulated that some other factor(s) are responsible for the chilling-induced respiratory rise. The mitochondria of plant tissues exposed to low temperature produce superoxide and/or hydrogen peroxide, when electron transport through the cytochrome pathway is impaired due to energy state of the cell or stress-induced physical changes in the membrane components. The alternate pathway of electron transport in the mitochondria, which is induced by low temperatures in some plant tissues can mediate these degradative processes by reducing the level of superoxide generated by the mitochondria.

The sucrose content remained unchanged till 60 h and started increasing at 72 h stage. Thus, the genesis of low temperature sweetening takes place after 3 days in case of Indian varieties. Consequent to sucrose accumulation, the reducing sugar content started increasing which could be ascribed to sucrose cleavage along with the stabilization in the respiratory activities. The sucrose content continued to increase showing continuous starch breakdown. Low temperature conditions result in accumulation of ATP in potato tissue. Changes associated with senescence are observed in tuber tissue exposed to low temperature, particularly in membrane systems, where peroxidation of lipids produces free radicals that contribute to further cell damage.

A possible defense mechanism to avoid free radical accumulation is the activation of the alternative pathway of respiration which consumes electrons diverted from the normal cytochrome pathway under low-temperature conditions. Evidence suggests that low temperature activation of the alternate pathway (cyanide resistant respiration) leads to decreased ATP levels and simultaneous increase in sucrose concentrations. The enzyme sucrose phosphate synthetase has been reported to play an important role in sucrose formation. This sucrose becomes the substrate for vacuolar acid invertase, resulting in the accumulation of reducing sugars. At lower temperatures of storage, invertase inhibitor is inactivated, leading to higher invertase activity causing cleavage of sucrose to reducing sugars. The statistically excessive reducing sugar accumulated somewhere between 12 and 17 days.

The reducing sugar content of above 100 mg/100 g fresh wt is considered unacceptable for making good quality potato chips. Thus, the specialized processing varieties namely Kufri Chipsona-1 and Kufri Chipsona-3 became unfit for chipping after 10 days of storage at low temperatures. As discussed above, sucrose formation at low temperatures and its subsequent cleavage to reducing sugars are catalyzed by the enzymes sucrose phosphate synthetase and invertase respectively. Therefore, the activities of sucrose phosphate synthetase and invertase in relation to low temperature sweetening in Indian potato genotypes need to be investigated. Xu et al have shown that total and basal activity of invertase has important bearing on the sugar accumulation during low temperature storage.

Level of anti-oxidants could also affect the cold-induced sweetening development in potato tubers. Phenolic compounds are one of the important contributors to the anti-oxidant capacity of potato tuber. The three varieties stored in this experiment had variable phenol contents, where Kufri Jyoti had the highest values. If reducing sugar accumulation after 35 days of storage in terms of number of folds over 0 day values was compared, the increase was 16-folds in Kufri Chipsona-1, 8-folds in Kufri Chipsona-3 and 4-folds in Kufri Jyoti. The mean values of phenolic content were also in the same order; however, further studies are required to reach at any valid conclusion.

Conclusion

The study has shown that the reducing sugars decrease in the initial phase of low temperature stress and then increase in the Indian potato processing varieties. While, the sucrose content remains static initially and then increase, when reducing sugar level becomes low. From the degree or number of folds increase in reducing sugars and
initial phenolics content, it appears that a possible correlation may exist between low temperature sugar accumulation and phenolic or anti-oxidant content of the tuber cell. Based upon the results of this study, it is suggested that respiration rate of tuber during storage, its anti-oxidant content and invertase activity should be considered in the development of new potato processing varieties for sub-tropical climates of India.

Acknowledgements
The author is grateful to Dr. S K Pandey, former Director, CPRI and Dr. B P Singh, Director, CPRI for providing the facilities and to Mr. Israr Ahmad and Mr. Santosh Mishra, students from GJU, Hisar, for excellent technical assistance.

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