

Histological and physiological changes of potato starch derived from seed and TPS (True Potato Seed) grown tubers under different cold storage duration

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Citation:

TAUFIQUE T., NISHIZAWA T., ROY T.S., MEIKO K., CHAKRABORTY R., MOSTOFA M., NARA K., 2021 - *Histological and physiological changes of potato starch derived from seed and TPS (True Potato Seed) grown tubers under different cold storage duration.* - Adv. Hort. Sci., 35(4): 371-381

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Data Availability Statement:

All relevant data are within the paper and its Supporting Information files.

Competing Interests:

The authors declare no competing interests.

Received for publication 25 July 2021

Accepted for publication 2 December 2021

Key words: Amylopectin, cold storage period, potato, starch granule size, sugar content.

Abstract: Starch granules in potato tubers exist with varying sizes and size distribution in nature. In this study, both the tubers of seed potato ('Lady Rosetta') and true potato seed (TPS) ('BARI TPS-1') varieties were stored at 5°C for 0 to 4 months, and the changes in the starch break down were analyzed physiologically and histologically to investigate how cold storage affects the starch break down. Although the starch content of both varieties reduced during cold storage, the reduction of starch content in 'BARI TPS-1' was higher than that of 'Lady Rosetta'. However, both volume and ovality (length:width) of starch granule did not change significantly throughout the storage period irrespective of variety, suggesting a non-uniform breakdown of starch granules. Scanning Electron Microscope (SEM) images of starch granule showed non-uniformed deformation and enlarged cavity or hole along the storage period, which indicated that starch breakdown occurred at a specific part of starch granule rather than peripherally and penetration would be deeper in 'BARI TPS-1' than that of 'Lady Rosetta'. However, there was no significant change in granule size distribution in spite of rapid degradation of amylopectin percentage in 'BARI TPS-1' than that of 'Lady Rosetta', suggesting more susceptibility of 'BARI TPS-1' to starch degrading enzyme and higher enzymatic action would cause deeper penetration in 'BARI TPS-1' than that of 'Lady Rosetta'.

1. Introduction

Potato (*Solanum tuberosum* L.) crop is usually cultivated by planting seed tubers which are genetically identical clones. On the other hand, True Potato Seed (TPS) is the actual botanical potato seed produced by the potato plant. Potato production from seed tuber derived

from TPS (seedling tuber) is emerging as a promising alternative of using seed tuber due to the advantages of less disease transmission, physiological maturity until planting season, cheap storage cost, and many choices of varieties (Pangaribuan, 1994). As potato tuber comes to maturity, starch-rich perimedullary region forms the major portion of the tuber (Gupta and Kaur, 2000), and potato granules are synthesized and stored as roughly spherical shapes in amyloplasts (Naeem *et al.*, 1997; Fajardo *et al.*, 2013). As the tuber matures, new starch granules are also synthesized in newly produced amyloplasts. There is usually a large granule size distribution within individual tubers in terms of percentages of small, medium, and large granules (Singh *et al.*, 2016). The size of small and large granules ranged from 0.6 to 6 μm and from 10 to 100 μm in potato starch, respectively (Wang *et al.*, 2018), consisting of smooth-surfaced, oval and irregular shape (Singh *et al.*, 2003). Singh *et al.* (2008) reported small starch granules of 1 to 10 μm , medium granules of 11 to 30 μm and larger granules of >30 μm in diameter in four different New Zealand potato cultivars.

Cold storage of potato tuber results in disintegration and disappearance of the amyloplast membranes around the starch granules, which bring in contact with the degradative enzymes such as α - and β -amylases and their substrates (Badenhuizen, 1965; O'donoghue *et al.*, 1995). Thus, prolonged storage of potato tuber at low temperatures can result in starch degradation and conversion of starch into reducing sugar (Zhang *et al.*, 2014). When potato tubers (*Solanum tuberosum*) are stored at temperatures below 9-10°C, the accumulation of sucrose and reducing sugars glucose and fructose occurred because of 'low-temperature sweetening' (LTS) (Pinhero *et al.*, 2007). The rate of starch degradation and sugar accumulation depends largely on cultivar and storage temperatures (Kazami *et al.*, 2000). Starch content was found to be decreased about 2 times after storage at 0-2°C for 8 weeks in both seed potato and TPS potato tuber by (Karim *et al.*, 2008). A decrease of starch content was also reported after 60-105 days of storage at 4°C in several Indian potato varieties (Yamdeu *et al.*, 2015). On the other hand, Biemelt *et al.* (2000) reported that the starch content did not alter throughout the storage period. The starch degradative enzyme not only affects the starch content in cold storage also starch granule. The enzymatic susceptibility of starch granules has been studied by various authors (Franco *et al.*, 1988;

Srichuwong *et al.*, 2005; Adejumo *et al.*, 2013). Differences in the enzymatic attack or susceptibilities of starches depend on many factors such as starch source, granule size, extension of association between starch components, rate of amylose and amylopectin, crystalline structure, particle size, surface porosity, type of enzyme (Hoover and Zhou, 2003; Kong *et al.*, 2003; Li *et al.*, 2004; Tester *et al.*, 2006). A shift to lower granule size distribution has been reported in raw starches of different potato varieties after in-vitro enzymatic hydrolysis (Kimura and Robyt, 1995). Cold Induced Sweetening (CIS) susceptible cultivars can cause smaller starch granules when stored for 4 or 12 weeks at 4°C rather than CIS resistant cultivars, which does not change until 24 weeks (Barichello *et al.*, 1990). Based on the previous observations, it was hypothesized that when potato tubers are stored at low temperature conditions, starch granule content and size change in relation to starch degradation. Starch granules may change randomly or according to size because several studies showed that the starch granule size is an important factor for influencing the digestibility of raw starch by amylase (Noda *et al.*, 2008). Ezekiel *et al.* (2010) observed that the number of small granules decreased, and the number of large granules increased in potato after cold storage. Granule size and surface area affect the hydrolysis rate of starch by amylase, large granules with higher diameter have smaller surface area than small granules with lower diameter and therefore larger granules digested more slowly (Tester *et al.*, 2006; Kasemwong *et al.*, 2008; Noda *et al.*, 2008). Because of the susceptibility to hydrolytic enzyme attack, deformation like external corrosion, pits or endo-erosion occurred on the starch granule (Planchot *et al.*, 1995).

Even though starch granules change in relation to starch degradation, it is unclear whether and how these happen in case of 'seed tuber' and TPS tuber at the same storage condition. So, the aim of this experiment was to study whether the starch degradation occurs similarly or differently in seed potato and TPS tuber; and how the starch degradation affects the starch granule deformation morphologically in both tubers.

2. Materials and Methods

Plant material

In October 2015, each of 50 potato tubers of

'BARI TPS-1' (TPS) and 'Lady Rosetta' (seed potato) were obtained from Bangladesh. Volume, size, and weight of all the tubers were measured and were stored at 5°C for 0 to 4 months in a refrigerator at the laboratory of the Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Bangladesh. The 'Lady Rosetta' variety is one of the widely cultivated commercial seed potato in Bangladesh, which is also used as processed potato. On the other hand, 'BARI TPS-1' is one of the well-cultivated 'True Potato Seed' variety, which is cultivated from true seed in first year and from the tubers from second year. Both of the tuber was collected and cultivated in the experimental plot of Sher-e-Bangla Agricultural University with integrated crop management. Ten tubers per each variety were taken out monthly from the refrigerator, the skin was peeled off and the tubers were grated using a grater of about 45 mm diameter. Half of the grated sample was then blended and washed with desalted water, and filtered through filter paper. This step was repeated 3 times and then filtrated and dried in sunlight under shaded condition for few days when the environmental temperature was around 34°C. This unheated samples were prepared for starch granule observation using SEM (Scanning Electron Microscope). The other half of the grated sample was added to 80% MeOH (100 mL) and heated at 80°C for 30 minutes. The supernatant was decanted, and the residue was extracted by 80% MeOH (100 mL). This step was repeated for 3 times in total and then washed by pure acetone. Then, it was heated and dried on a hot plate at 50 to 70°C only in the daytime and stored at the ambient temperature at night. It took 3 days to dry completely and was weighed repetitively up to constant dry weight. The completely dry sample was cooled to the ambient temperature, stored in a freezer at -20°C. This 80% MeOH (100 mL) extracted samples were used for sugar and starch analysis. The chemical analysis was done at the laboratory of the Department of Bioproduction, Faculty of Agriculture, Yamagata University, Japan.

Soluble sugar content

A 0.2 g of dry powder from each cultivar was taken into a test tube, then 9 mL of 80% MeOH was added, and it was heated at 80°C for 30 min. The extract was centrifuged at 3000 rpm using a centrifuge (KS-500, KUBOTA, and Tokyo) for 10 min, and the supernatant was decanted. This extraction procedure was repeated for 3 times, and the combined

supernatant was made up to 50 mL volume with 80% MeOH. Reducing sugar content in 0.5 mL of the solution was measured by Somogyi Nelson method (Nelson, 1944), and the 0.5 mL of the solution was added by 2 units of invertase (pH 4.5, Kanto, Kagaku, Tokyo) and hydrolyzed at 50°C for 30 min. Sucrose content in the solution was also measured using the same technique as reducing sugar. Copper reagent and nelson reagent were used to prepare a standard solution of glucose. Absorbance was measured at 660 nm, and a standard curve was prepared to calculate reducing sugar. Non-reducing sugar was measured from hydrolytic degradation of sucrose, and absorbance was measured at 660 nm.

Abs obtained from the analysis of reducing sugar was denoted by ABS 1 ... (1)

Abs obtained from the analysis of non-reducing sugar was denoted by ABS 2 ... (2)

Concentration of non-reducing sugar was calculated from = {Abs (2) - Abs (1)} × 0.95 ... (3)

Concentration of total soluble sugar was calculated from = (1) + (3)

These steps were repeated for 5 times for each cultivars and for each storage sample.

Starch content

The insoluble solid from the 80% MeOH extract was added with 1.5 mL of distilled water and heated at 100°C for 1 hr. Starch in the pellet was hydrolyzed using amyloglucosidase (Yakult) at 55°C for 3 hrs then neutralized by 0.1% NaOH solution. Starch content was also measured in 0.5 ml of the solution using the same technique of sugar analysis and Glucose Oxidase method. Glucose standard curve was prepared to measure starch content. Absorbance was measured at 660 nm for Somogyi Nelson Method and at 500 nm for Glucose Oxidase method and was repeated for 5 times for each cultivars and for each storage sample.

Abs obtained from the analysis of starch was denoted by Abs ... (4)

Starch content was calculated from = Abs 4 × 0.9.

Histological analysis of starch granule

Starch powders were scattered on an adhesive carbon tape and were fixed with 2% osmium tetroxide (OsO₄) and successively washed with 50 mM cacodylate buffer and ultrapure water. The dried samples were coated by Pt using each sample slides by Pt ion coater (JFC-1200, JEOL, Tokyo), and then observed under SEM Scanning Electron Microscope (SEM, TM3000, Hitachi, Tokyo). The length and width

of the starch granules were measured by using Motic Image Plus 2.0 software from SEM images. The length and width ratio of starch granule was measured as the ratio of length:width.

Starch granule was regarded as an ellipsoid, and the volume of starch granule was measured of each axis of the ellipse. For histogram analysis (volume and length/width ratio) 10 SEM image of x 500 magnification and 200 µm across from each treatment were chosen and 5 granules from each image were measured randomly (total 500 starch granules).

Amylose percentage determination

Amylose content was measured using an assay kit (Megazyme, Amylose/Amylopectin Assay Kit, Ireland) according to the procedure outlined by the manufacturer. Percentage of amylose was directly calculated following the specific Megazyme equation based on the measured absorbance values, no additional standard curve or equation was generated for this study. Amylopectin content was calculated by 100% difference of the amylose content (Aristizábal *et al.*, 2007). This step was repeated for 5 times for each cultivar and for each consecutive storage sample.

Statistical analysis

Data were subjected to analysis of variance and the difference between cultivars was compared with t-test using SPSS software.

3. Results

Soluble sugar content

In 'BARI TPS-1' tubers, reducing sugar content reached the highest value (57.80 mg/g DW) after 1 month of storage then decreased rapidly thereafter. Reducing sugar of 'Lady Rosetta' showed the same tendency but the highest value after 1 month of storage (25.86 mg/g dry weight) was less than half of that of 'BARI TPS-1' and decreased gradually thereafter (Fig. 1). Therefore, reducing sugar content of 'BARI TPS-1' was significantly higher than that of 'Lady Rosetta' during 1 to 3 months of storage. Although, sucrose content also showed the highest value after 1 month of storage, the value decreased rapidly thereafter. The decline of 'BARI TPS-1' was slower than that of 'Lady Rosetta', resulting in a significant difference between the two varieties during 2 to 3 months of storage (Fig. 1). Changes in total sugar content were similar to that of sucrose content and significantly higher content was observed also in

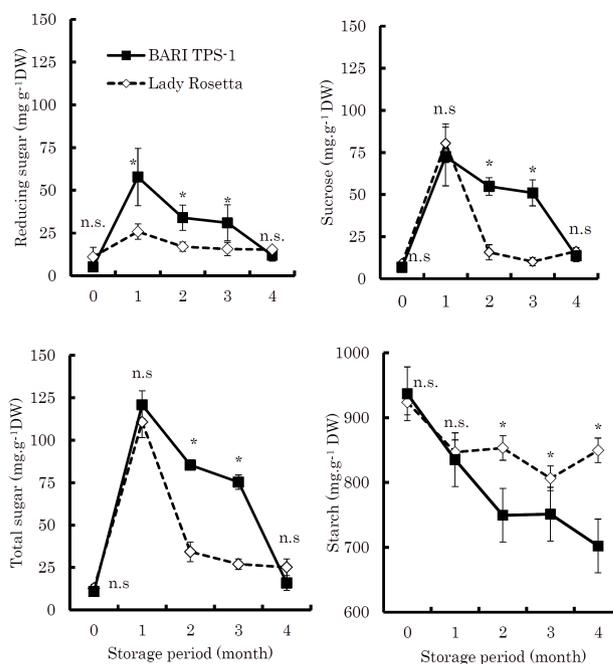


Fig. 1 - Changes in soluble sugar and starch contents of two potato cultivars as affected by different storage period (n = 5 ± SE). * shows significant difference and n.s. shows non-significant different at P<0.05 by student's t-test.

'BARI TPS-1' during 2 to 3 months of storage (Fig. 1).

Starch content

Although starch content of both potato varieties decreased continuously throughout the storage period, starch content of 'BARI TPS-1' decreased more rapidly than that of 'Lady Rosetta' variety, resulting in a significant difference from 2 to 4 months of storage (Fig. 1). It is noticeable from figure 1 that decreasing level of starch content was on par with the increasing reducing sugar content, where 'BARI TPS-1' showed rapid starch degradation with higher reducing sugar content after 2 to 4 months of storage than that of 'Lady Rosetta'. Similarly increase in sucrose level of 'BARI TPS-1' after 1 month of storage paralleled with the decreased level of starch content, resulting significant differences between two varieties.

Starch granule sizes

Starch granule length, width, length:width ratio and volume did not change apparently throughout the storage period. Though 'Lady Rosetta' had a tendency to have slightly higher values in length, width and volume, there was no significant difference between the both 'BARI TPS-1' and 'Lady Rosetta' (Fig. 2).

Histogram was shown for starch granule volume

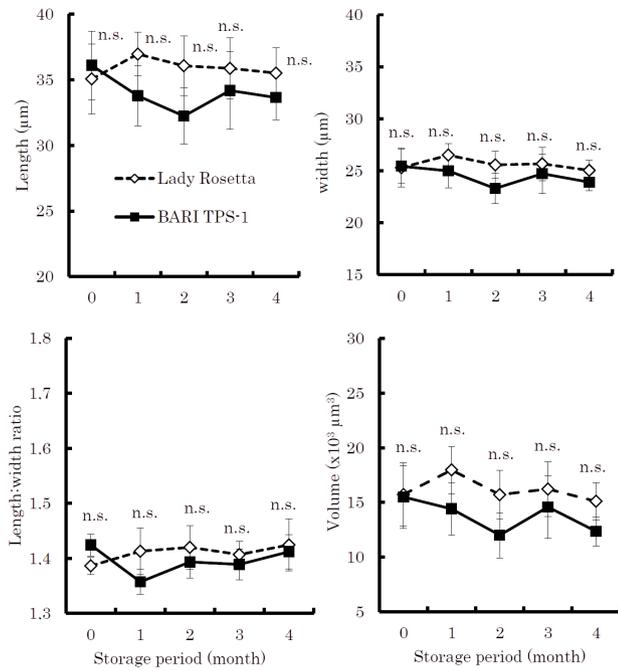


Fig. 2 - Changes in the sizes of starch granule derived from two potato cultivars as affected by different storage period for (n =10±SE). *shows significant difference and n.s. shows non-significant at P<0.05 by student's t-test.

and ovality (length:width) within the range of (0.5 to 81.5) x 10³ µm³ and (1.05 to 2.22) respectively (Fig. 3 and 4). The highest frequency was observed at (7.6 - 13.5) x 10³ µm³ and (1.19 and 1.31) for volume and ovality, respectively and there was no considerable differences between the two cultivars. Moreover, no apparent change also occurred during storage period.

Starch granule morphology

Both 'BARI TPS 1' and 'Lady Rosetta' tubers showed normal and smooth starch granule surface at 0-month storage (Fig. 5). Starch granules of both tubers changed after storage condition. Pit and hole like structures were observed after 1 month storage in both varieties. After 2nd, 3rd and 4th month storages prominent depression or cavity was observed in both potato cultivars. However, both potato tubers showed a similar pattern of deformation.

Amylopectin percentage

Although the percentage of amylopectin in both potato varieties decreased continuously throughout the storage period, Amylopectin of 'BARI TPS-1' decreased more rapidly than that of 'Lady Rosetta' variety, resulting in a significant difference from 2 and 4 months of storage (Fig. 6). Although 'Lady Rosetta' variety had slightly higher percentage than TPS after 3 months of storage, there was no significant difference between them.

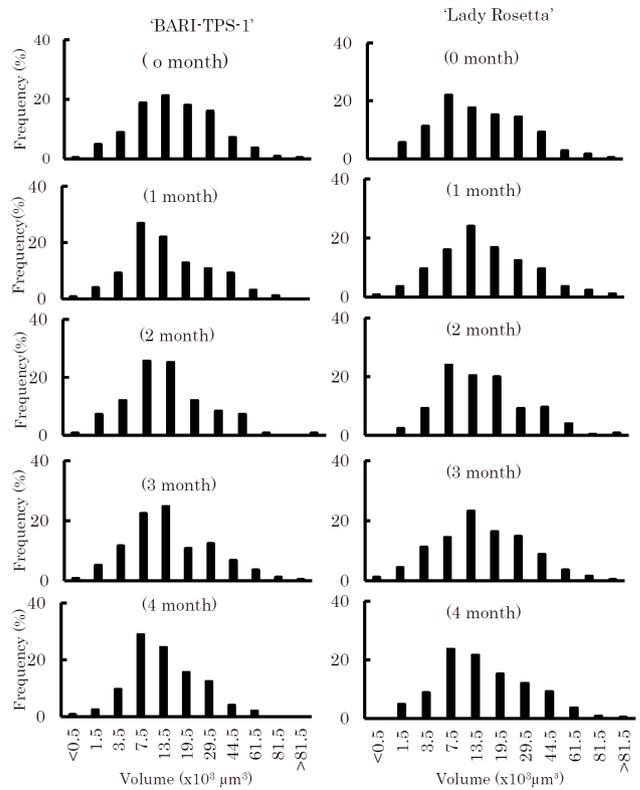


Fig. 3 - Change in histogram for frequency percentage of potato starch granule volume derived from 'BARI-TPS-1' and 'Lady Rosetta' potato varieties as influenced by storage period.

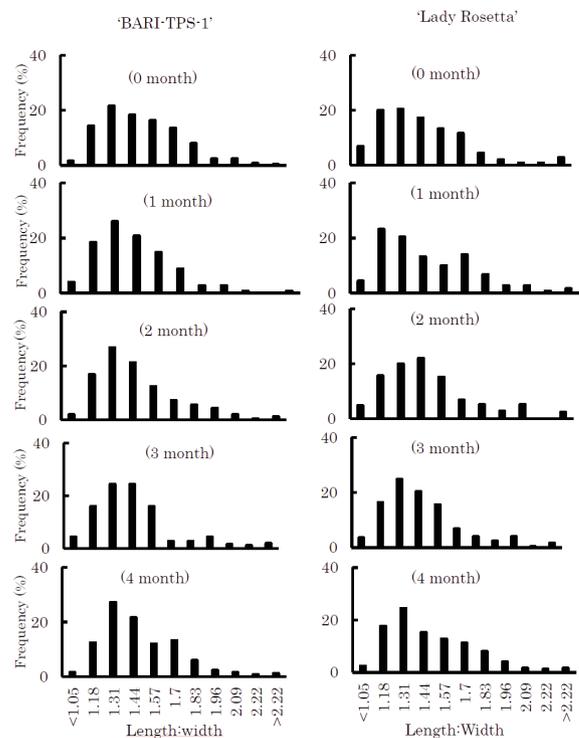


Fig. 4 - Change in histogram for frequency percentage of potato starch granule Length:Width ratio derived from 'BARI-TPS-1' and 'Lady Rosetta' potato varieties as influenced by storage period.

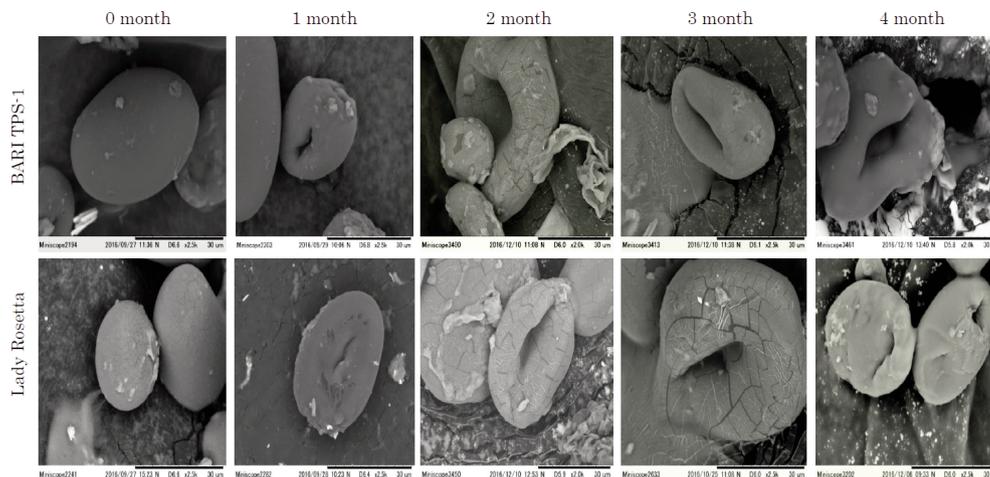


Fig. 5 - Scanning electron micrographs showing the surface morphology of starch granules of 'BARI TPS-1' and 'Lady Rosetta' and potato tuber stored at 5°C at 0 to 4 months of storage.

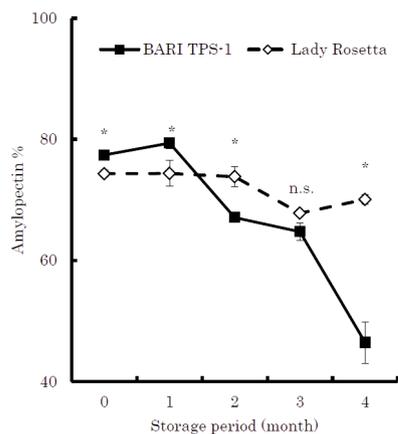


Fig. 6 - Changes in the percentage of amylopectin in 'BARI-TPS-1' and 'Lady Rosetta' potato tubers as influenced by storage period.

4. Discussion and Conclusions

In potato tuber, starch is converted to sugar during cold storage (Malone *et al.*, 2006). The starch content of potato tuber decreased markedly during prolonged storage at 4-8°C through the process of conversion of starch into sugars (Nourian *et al.*, 2003; Smith *et al.*, 2005). Ohad *et al.* (1971) also reported reduction of starch content by 26% after 17 days of storage. Mature dormant potato tuber produced sugars by degradation of a small fraction of starch (Isherwood, 1973). Thus starch content decreased because of the hydrolysis of starch by starch degrading enzymes (Nielsen *et al.*, 1997), suggesting why

reducing sugar content increased as starch content decreased during cold storage (Fig. 1). Storage at 0-5°C increases sugar accumulation in potato tuber (Wismer *et al.*, 1995; Blenkinsop *et al.*, 2003), which coincides with these experiments where both varieties showed higher reducing sugar content after storage with a significant difference between 'BARI TPS-1' and Lady Rosetta (Fig. 1). This increased level of reducing sugar and decreased level of starch content is related with the increased activity of hydrolysing enzyme (Sowokinos, 2001), which might explain the higher starch degradation in 'BARI TPS-1' (Fig. 1).

During low-temperature storage, the enhancement of sucrose and hexoses (glucose and fructose) levels is known as "cold sweetening" or 'low temperature sweetening', which is an important metabolic process in the roots of many species as well as potato tuber (Wismer *et al.*, 1995; Espen *et al.*, 1999; Galindo *et al.*, 2004; Galindo *et al.*, 2007). The soluble sugar content of potato tuber increases at low temperature such as 4°C, when stored for 6 to 12 weeks because of starch decomposition and then inclines to decrease (Cochrane *et al.*, 1991; Chen *et al.*, 2012). Total sugar and sucrose content both showed a similar changing pattern after storage with significantly higher content in 'BARI TPS-1' during 2 and 3 months (Fig. 1). Cold storage condition triggers the tuber starch to breakdown into sucrose through various hydrolytic enzymes which further hydrolyzed into reducing sugars (glucose and fructose) (Sowokinos, 2001). The amount of soluble sugar accumulated in

tuber also depends on different cultivars (Zommick *et al.*, 2014).), which could explain the higher reducing sugar in 'BARI TPS-1' after storage than Lady Rosetta (Santos *et al.*, 2020).

However, there was a sudden increase in starch content between 3rd to 4th months of storage in 'Lady Rosetta' variety (Fig. 1), suggesting recondition of starch or increase of respiration losses during storage. The difference in respiration rates depend on cultivar, growing conditions, experimental conditions, and physiological status of the tubers (Fennir *et al.*, 2003). Comparatively lower starch degradation after 3 to 4 months of storage might have contributed to lower presence of reducing sugar in 'Lady Rosetta' (Fig. 1). This can also be explained that likely the rise of starch content between 3 and 4 months of storage, amylopectin percentage also showed similar kind of tendency in 'Lady Rosetta' variety (Fig. 6). An increase in amylopectin percentage from 3 to 4 months of storage suggested lower enzymatic degradation (Fig. 6) (Hofvander *et al.*, 2004). It seemed amylase enzyme activity reduced during 3 to 4 months of storage because of sprouting of seed potato varieties or it can be explained that the amylase enzyme activity was not enough to degrade the starch in seed potato (Lewis *et al.*, 1994). As the experimental condition was same for both of the tubers, hence these may explain faster physiological aging in seed potato variety than TPS variety. This may also suggest that experimental storage temperature might have affected the respiration losses; and the sugar had been used for tuber germination in case of seed tuber (Olsen *et al.*, 2003).

The TPS variety showed higher starch degradation rate than the seed potato (Fig. 1), and sharp decrease in amylopectin percentage after storage (Fig. 6), moreover there was higher percentage of amylopectin degradation in 'BARI TPS-1' than 'Lady Rosetta' starting after 1 month of storage and continued as the storage progressed (Fig. 6), but there was no apparent changes in starch granule volume and ovality (length:width ratio) (Fig. 2). This result suggested a non-uniform degradation pattern of starch granules in 'BARI TPS-1' and 'Lady Rosetta' varieties.

Histograms were also carried out to investigate, if the starch granule deformation primarily started in smaller sized granules, or larger sized granules (Figs. 3 and 4). Singh *et al.* (2008) reported a shift of granule size range to smaller granule sizes in isolated starches of New Zealand Taewa (Maori potato) when they were stored for three and six months at 4°C

temperature. This experiment also reported erosion and pitting on the surface of stored potato starch. In case of banana, surface erosion by enzymatic degradation resulted in smaller granules with elongated shape and exo-corrosion process caused pits on the surface of the starch granule with high frequency (Peroni-Okita *et al.*, 2013). This shift of granule size distribution has been related to rapid digestion of starch granule and composition variation between small and large granules (Salunkhe *et al.*, 1989). However, there were no considerable changes of starch granule sizes in both potato cultivars in our study. This suggested that, in spite of having significant change in starch degradation along storage in both variety, it had hardly influenced the size of starch granule (Fig. 2, 3 and 4).

Moreover, it was found that even after 4 months of storage periods, there was no significant change in starch granule sizes in both cultivars, in spite of 'Lady Rosetta' having slightly higher tendency in the value of length, width and volume (Fig. 2). Fajardo *et al.* (2013) also reported unchanged granule size after storage, which was attributed to the nearly undetectable change in volume of starch granule. Russet Burbank potato tubers also showed similar granular size distribution after storage at 3.9°C (Johnston *et al.*, 1968).

SEM observation showed that starch granules from both harvested tubers presented smooth granular surface under SEM without any storage treatment or at 0-month storage (Fig. 5). Although there is argument about the appearance of natural potato starch granular surface, several studies showed smooth granular surface of natural potato starch under microscopic observation. Cottrell *et al.* (1993) reported smooth granular surface of potato starch of Record and Brodick potato cultivar at harvest. (Sarıkaya *et al.*, 2000) also reported smooth granular surface of potato starch before any enzymatic or freezing treatment. As the SEM observation in this experiment showed hole or pit formation on granular surface of both varieties after storage (Fig. 5) (Sarıkaya *et al.*, 2000; Noda *et al.*, 2005), it is suggested that the long term storage at low temperature of this experiment lowered the starch content which led to susceptibility to hydrolytic enzyme attack that changes the properties and composition of starch granule (Barichello *et al.* 1990). The cold storage temperature might have given damage to amyloplast membrane by starch hydrolysis (Ohad *et al.*, 1971), which enhanced membrane permeability through

starch hydrolysis enzymes, and resulted the formation of cracked region or surface hole on the granule (Sujka and Jamroz, 2010).

However, no apparent change was observed in the ratio of granules and histogram analysis of both potato starch granule, but the SEM observation clearly showed deformation of starch granule forming hole or pit like structures gradually started from 1 month of storage and continued to 4 months of storage (Fig. 5). On the other hand, gradual starch degradation and decreased amylopectin percentage indicated activation of enzymatic degradation in both cultivars after storage (Fig. 1 and 6). Therefore, it is suggested that starch granule erosion may occur at specific surface region of the granule causing no change in the granular size. Starch hydrolyzing enzyme like α -amylase might have attacked at particular points on the granule surface, forming tunnels into the granule, thus hydrolyzed the granule from the inside (Lindeboom *et al.*, 2004). Similar finding was reported by Peroni-Okita *et al.* (2013) where starch granules of green banana maintained the rounded shape after low temperature but presented pits on the granule surface which was enlarged by the corrosion process, suggesting partial degradation of starch granules.

Thus enzyme molecules can affect the starch granule in different patterns either by forming pin holes, medium sized hole, sponge like erosion or selected point at the surface leading to a single hole (Sujka and Jamroz, 2010). Enzyme can gain access to innermost region of the starch granule by faster digestion than at the periphery region of the granule, which can form shallow hole like structure (Duffus, 1984). This may explain how the both starch granules showed no changes in their sizes despite of granular deformation.

However, 'BARI TPS-1' showed a higher starch degradation percentage throughout the storage period than the seed tuber potato. This suggested higher susceptibility of starch degrading enzyme in TPS tuber than that of seed potato tuber. Variation in starch granule morphology and their crystalline organization may explain the susceptibility to enzymatic degradation (Gallant *et al.*, 1992). Amylase plays the major role in in-vivo breakdown of starch (Manners, 1985). Amylose and amylopectin ratio may explain the degradation pattern of starch granules in both TPS and seed potato varieties. Both the potato tuber showed decreased percentage of amylopectin along the storage period, whereas 'BARI TPS-1' resulted

lower amylopectin percentage than the seed potato (Fig. 6). Bach *et al.* (2013) described that as amylopectin degrades more rapidly than amylose, it causes activation of more starch degrading enzymes. Therefore, 'BARI TPS-1' might have allowed deeper penetration of starch degrading enzymes than the seed tuber in spite of no apparent changes in granule size (Fig. 5). As the extent of pit or cavity of starch granules were not determined in this experiment, it can be suggested that probably a different inner molecular organization of TPS starch granule may be allowed differentiated enzyme attack, which could be responsible for slightly smaller starch granule than 'Lady Rosetta' during Storage (Fig. 6) with no significant difference. This also suggests that starches vary in their resistance to enzymatic susceptibility (Srichuwong *et al.*, 2005).

There are different opinions about the presence of pores or holes on the surface of potato starch granules. Although several observations concluded that some factors could cause an increase in the number and size of pores on starch granules surface (Fannon *et al.*, 1992). Moreover, the expansion of granule degradation increased with the increased enzyme concentration (Mu *et al.*, 2015). During enzymatic hydrolysis, some regions of granules are more susceptible to enzyme attack because of less organized amorphous rings, whereas the crystalline lamella provides higher resistance to enzymatic erosion (Oates, 1997). This kind of enzymatic hydrolysis was characterized by forming a hole by creating channel through the less resistant granule core (Jung *et al.*, 2017), which may explain deeper enzyme penetration in TPS tuber. Action pattern of both endo- and exo-amylase at a lower temperature may explain overall amylolytic activity in TPS and seed potato tubers (Shin *et al.*, 2002; Nabubuya *et al.*, 2012).

4. Conclusions

In this experiment, the starch content of TPS tuber degraded more rapidly and produced higher reducing sugar content than the seed potato variety after storage. This result indicated that, TPS tuber may not be acceptable for low temperature storage compare to seed potato variety. There were no significant changes in granule size and volume, indicated that granule did not change as a ratio in both tubers. However, both potato granules deformed by forming holes or cavities at innermost surface region

after storage, suggesting the starch granule degraded partially rather than concentrically. As both the tubers showed a similar kind of degradation pattern of starch granule in spite of higher starch degradation rate in TPS tuber, similarly the rapid degradation rate of amylopectin in TPS tuber explained higher amylase activities in TPS than the seed tuber. This result suggested possibilities of deeper penetration in TPS starch granule than the seed tuber starch granules. Further studies on the susceptibility of starch degrading enzyme on TPS and seed potato can be done to foresee the starch degradation pattern in TPS and seed potato tuber.

Acknowledgements

The authors thankfully acknowledged the authority of Japan Society for Promotion Science (JSPS) and University Grants Commission (UGC) of Bangladesh for supporting to conduct of the experiment.

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