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Starch Granule Sizes of Surface and Inside Tissues of Potato Strips after Deep-Freezing Storage

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ABSTRACT

It is a common practice in plant research that fresh plant samples are stored in -80°C after rapid freezing in liquid nitrogen. However, the effect of this process on plant tissues is unclear. Most plant tissues have starch granules. We conducted microscopic observation of starch granules from both the surface and inside of potato tuber strips of the cultivar 'Shepody' one hour as well as 5-years storage at -80°C after the liquid nitrogen deep-freezing. The starch granule shape is similar before and after the two storage durations. After one hour storage, the starch granule length showed no changes. However after the long-term storage, the surface ones were significantly shorter than those from the inside tissues of the strips. The mean length of all the granules was 24 and 28 µm, respectively, for starches on the surface and inside, while the mean length of the 10% largest starch granules was 45 and 57 µm, respectively. The reduction in length of the surface starch granules is likely due to potential water evaporation inside the closed plastic tubes. The results indicate that native fresh potato starch can reduce its granule size under certain conditions. This finding might open approaches to adjust starch-related oil absorption during frying of frozen potato products.

Keywords: microscopic analysis, potato tuber, starch granule length

INTRODUCTION

Deep-freezing storage, usually with rapid freezing in liquid nitrogen first and then storage under -80°C, is widely used in plant material storage for various analyses later in cellular and molecular biological research such as in organelle isolation and enzymatic extraction, assuming the biochemical properties of the materials are unchanged. Rapid deep freezing has been also used in preparation of tissues for various microscopic observations (Umrath 1974; Bechtel and Wilson 1997). Although rapid deep-freezing followed by -80°C storage is very convenient for experimenters to focus on materials preparation during sampling, it is actually not very clear to what extent the process may modify the properties of the materials. Starch granules can serve as a model for evaluating the potential effects of the deep-freezing and storage since starch granules are an important part of most plant tissues and also are the targeted harvest component for food crops. Multiple freezing/thawing treatments influenced the gelation characteristics, water solubility and water holding capacity of commercial dry starch granules (Szymonska *et al.* 2003). However, it is unclear whether native starch granules sizes changes during the deep-freezing treatments and storage.

In this study we investigated the effects of deep-freezing of potato strips on their starch granule sizes in comparison between starch granules on the surface and those inside the strips.

MATERIALS AND METHODS

Potato materials

All the potato tubers in this study were from the cultivar 'Shepody' stored at 4°C and approximately 90% humidity. The potatoes were from the years 2002 or 2008. The 2002 potatoes were from plants growing in a sunlight screening nursery. The 2008 potatoes were from the field at the Agriculture and Agri-Food Canada's Benton Potato Breeding SubStation in New Brunswick.

Deep-freezing process

The tubers from the 4°C storage were cut into small strips about 0.5 × 2 cm in size. The strips were put into 12 ml polypropylene tubers (Cat# T406-2A, Simport Plastics Ltd, Beloeil, Canada) to a level about 80% full and immediately frozen in liquid nitrogen. The plastic tubes containing the strips were then tightly closed with caps and stored at -80°C. The 2008 potatoes were analyzed one hour after being deep-frozen, and the 2002 tubers five years after deep-freezing. Note that water does not leak out from this type of plastic tube when closed with the cap.

Microscopic measurement of starch granule sizes

The surface juice with starch granules were taken by slightly scratching the surface tissues of the strips using a small blade (Ref. P308, Lance Paragan Ltd., Sheffield, England). The surface of the strips thawed during this scratching. Then each strip was cut into two equal size pieces, and the juice from the inside tissues was taken using the same method. The juice was mixed with 9 µl distilled water in a round chamber (6 mm in diameter) on a microscopic slide, and then covered with the covers glass on the top.

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Short Communication

Granule observation was under a microscope (Carl Zeiss, Inc, Germany) with a 20X Epiplan objective. The starch granule measurement was with the Zeiss software AxioVison Rel.4.7. with manual labeling of the maximum length of each starch granule. Three tubers were used separately for each treatment for studying the effects of short-time deep freezing. Two potatoes were used separately for studying the effects of long-term storage. The microscopic procedure was repeated seven times, each with one strip. The ANOVA and Duncan multiple means comparison were conducted using the SAS program, version 9.2. Details of the microscopic measurement method will be published elsewhere.

RESULTS AND DISCUSSION

The lengths of starch granules were measured from the surface and inside strips one hour after frozen, as well as from the control (immediately after striping before frozen). There was no significant difference found between starches of these three sources. The mean length of all the starch granules of the three starch sources was 24 µm, and the grand mean of the length was 52 µm for the 10% largest granules (**Table 1**). Given to the resolution limit of the technique, changes of starch in fine structure level cannot be ruled out. For example, it has been reported that after the fifth time of deep-freezing of dried starch, a well-ordered microstructure of the surface with distinct straight chains of fine particles of about 30 nm in diameter could be observed (Szymonska *et al.* 2003). In the present study, the results indicate that the deep-freezing treatment did not significantly change the size, in terms of length, of the starch granules even through the fresh starch granules are expected to have a certain amount of water inside the granules.

For the long-term stored strips, the mean length of starch granules was significantly different ($P < 0.01$) between starch granules on the surface and those inside the strips (**Table 2**). The results were consistent for both tubers analyzed. Even though the two tubers also had significant differences in starch granule length (21.56 µm vs. 27.80 µm), the grand mean of all the starches on the surface was 21.04 µm whereas that of the starches inside was 28.13 µm. The shape of the starch granules had no obvious difference between the ones isolated from surface tissues and the ones from inside tissues despite the size changes (**Fig. 1**).

For the 10% largest starch granules, in terms of length, from the long-term stored potato strips, the starch granules in the surface tissues of the strips was also consistently smaller than the ones inside, with mean lengths of 44.88 µm and 60.30 µm for those on the surface and those inside, respectively (**Table 3**).

It has been reported that water can be identified inside native potato starch granules in the amorphous growth rings, the semi-crystalline lamellae and channels (Tang *et al.* 2000). Therefore, the size reduction for the fresh starch granules in the surface tissues might be due to the potential water loss during the long-term storage inside the storage plastic tubers, even though the caps of the plastic tubes were well closed. To the best of our knowledge, this might be the first report of a significant size reduction of fresh native starch when the potato tissues lost some water.

For cellular and molecular research of deep-frozen tissues, this study suggests that researchers should be alert to the changes caused by the deep-freezing storage of plant samples, although deep-freezing of plant samples is one of the best methods in storage of plant samples. Potato strips have relatively smaller surfaces compared to leaves. It is expected that leaf sample analysis might be even more affected by the surface/inside differences. If starch granules can change size, other cell structures might also have changes, which may affect various analyses.

It is expected that water contents inside and outside of starch granules can have impact on the potato boiling and tuber strip frying quality. Smaller granules may have smaller surface areas per granule, compared to large granules. It has been reported that the presence of water was crucial for starch swelling and that dry granules remain almost intact after exposure to hot oil (Aguilera *et al.* 2001). Oil absorption is likely be affected by the surface areas. Therefore, the starch granule size difference between surface starch and inside starch might be relevant to potato processing quality. The finding of size reduction of fresh native starch in certain conditions may open new approaches to reduction of frying-related oil absorption through surface treatment of potato chips/strips. Studies on the biochemical and biophysical properties of granules should provide further insights of the freezing effect on these subsequent potato processings. The effects of medium-term storage and other factors such as cultivars and different storage temperatures need to be further investigated.

Table 1 Effects of freezing in liquid nitrogen and subsequent storage under -80°C for one hour on potato starch granule length.

Treatment	No. of starch granules	Grand mean (µm)*	Mean of the 10% largest granules (µm)*
Frozen: Surface	1638	24.41 A	52.05 A
Frozen: Inside	2013	24.35 A	51.67 A
Control (Before freezing)	2081	23.77 A	53.11 A
Grand mean	1911	24.04	52.29

*Values with the same letter within the same column are not significantly different ($P < 0.01$) according to the ANOVA and Duncan's multiple-range test. The means were from three separately measured tubers.

Table 2 Comparison of starch granule length[#] between surface and inside parts of the potato tubers.

Tuber	Granules scored (No.)		Surface granule length (µm)**	Inside granule length (µm)*	Grand mean (µm)**
	Surface	Inside			
Tuber 1	1070	457	19.46	25.47	21.56A
Tuber 2	463	699	24.67	29.87	27.80B
Grand mean	767	578	21.04A	28.13B	24.09

[#]Only granules larger or equal to 8 µm were included in the analysis.
**: Values with different letters within the same row or column are significantly different at $P < 0.01$ level according to the ANOVA and Duncan's multiple-range test.

Table 3 Comparison of 10% largest granules between surface and inside parts of the potato tubers.

Tuber	Granules scored (No.)		Surface granule length (µm)**	Inside granule length (µm)*	Grand mean (µm)**
	Surface	Inside			
Tuber 1	100	46.00	40.00	51.17	43.52A
Tuber 2	46	70.00	55.48	60.30	58.39B
Grand mean	73	58	44.88A	56.68B	50.10

**: Values with different letters within the same row or column are significantly different at the $P < 0.01$ level according to the ANOVA and Duncan's multiple-range test.

CONCLUSIONS

Fresh native starch granules of potato strips do not have significant changes in size if the deep-freezing storage is short, but the surface granules reduce their sizes after long-term storage. These findings of size changes in fresh native starch under certain conditions could have practical applications in adjustment and/or prediction of quality of frozen potato products after medium or long-term storage.

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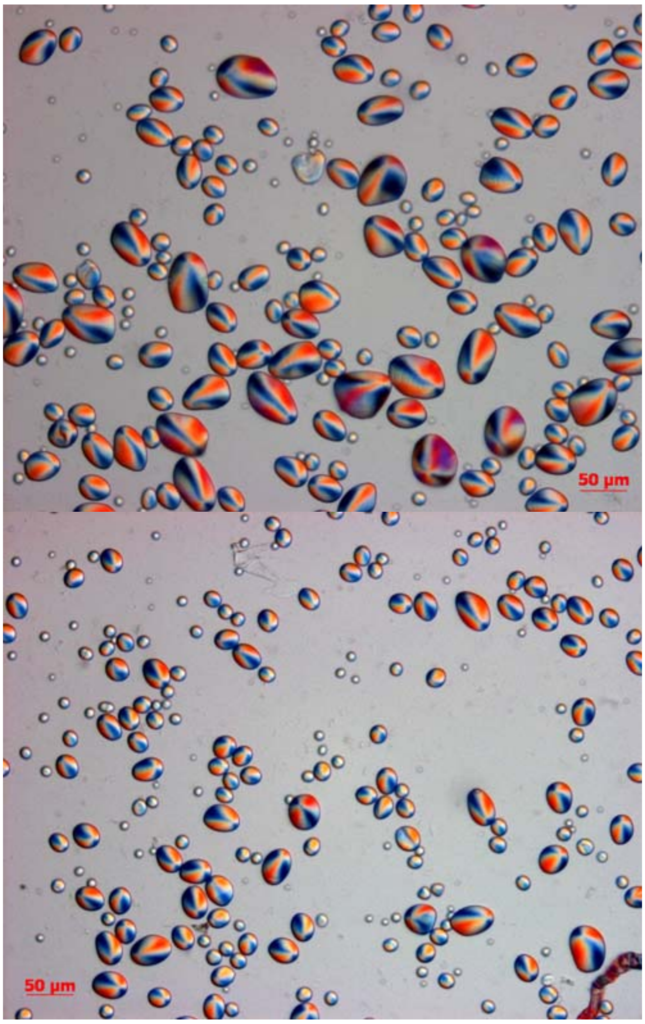

Fig. 1 Native fresh starch granules of long-term deep-frozen potato strips. Top: from the inside of the strips; Bottom: from the surface of the strips.

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