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THE EFFECT OF IRRADIATION ON STARCH CONTENT IN GOLDEN DELICIOUS APPLES

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Abstract

Starch content in apple (Golden Delicious) skin and flesh was studied as a function of radiation dose (0, 0.5, 1, 2 and 5 kGy) after 8 weeks shelf life (16°C, 80-90% RH).

Starch was generally not found in the flesh with the exception of the 5 kGy sample. Starch grains were observed, however, in plastids of the epidermis and especially in the hypodermis in correlation with the radiation dose. 1 kGy dose caused a significant effect (P < 99.98%) on retardation of starch breakdown measured by electron microscopic morphometry and chemical analysis.

Analysis showed that increasing radiation dose increased glucose concentration in the flesh. In the skin the concentration of all three sugars, glucose, fructose and sucrose, increased with irradiation up to 2 kGy, but they decreased with higher doses.

Introduction

Starch begins to accumulate in a developing apple fruit 3 to 4 weeks after bloom. Over the next 2 months it accumulates to a maximum value and subsequently declines as the fruit matures. Starch synthesis and decomposition are modified by different circumstances. Starch is a reserve carbohydrate that is eventually quantitatively converted to soluble sugars, that are stored in the vacuole or metabolized by the cell through respiration.

Simons and Chu (1982; 1983) investigated corking, a physiological disorder of apples. Cytoplasmic membrane breakdown occurred in cortical cells adjacent to the vascular bundles at least 45 and 60 days prior to fruit maturation. Cell walls were thick in fruit exhibiting corking. Starch accumulation occurred within localized areas and was attached to the cell walls. Starch grain accumulation on cortical cell walls and proliferation of starch grains in cells having thick walls was noticed 30 days before maturation (Simons and Chu, 1982; 1983). Profiles of tissue development and subsequent breakdown indicate that this physiological disorder has developed in formative and developmental stages of growth, with extensive tissue breakdown. Electron microprobe studies provided evidence that both potassium and calcium concentrations were low in all affected tissues, although the potassium level was higher than calcium in most of the sampled areas (Simons and Chu, 1982).

Mahanty and Fineran (1975) investigated the cellular ultrastructure of control and calcium sprayed apples which were cool-stored for 3 months. Cells of the control sample were less well preserved compared to those of calcium sprayed apples. Chromoplasts of the epidermis had prominent plastoglobuli and thylakoids which were scattered or sometimes alveolar rather than compacted. Cells from hypodermis showed increased lysosomal activity. Mitochondria and
plastids containing starch appeared normal. In the chromoplasts of the calcium sprayed apples, compacted thylakoids and plastoglobuli were present. The inner cortical cells had a large central vacuole, and the cytoplasm surrounding this was usually granular and contained numerous plastids with starch. Gamma-irradiation has been reported to increase the sucrose content of potato tubers and sweet potato roots (Hayashi and Kawashima, 1982a, b). Sucrose accumulation was dependent upon irradiation dose, and the preferable dose range was between 1-2 kGy for roots of sweet potato. The sucrose content of irradiated vegetables and fruits increased for a long period after irradiation. It was suggested that an enhanced activity of sucrose synthase and sucrose-phosphate synthase played an important role in the sucrose accumulation in irradiated potatoes (Hayashi and Kawashima, 1983; Hayashi and Aoki, 1985).

Kovács and co-workers (1988) investigated the microstructure of calcium treated and irradiated apples, a short time after treatment and after 3 months subsequent cool storage. All treatments (calcium, irradiation, calcium treatment combined with irradiation) maintained the basic compartmentation of the cells into cytoplasm, plastids and vacuoles. By contrast, and mainly in the epidermis, the integrity of vacuoles had been lost in the control by the end of storage. Calcium treatment either alone or in combination with irradiation resulted in a somewhat better preservation than irradiation alone. Irradiation and combined treatment preserved starch. The difference was evident already 4 days after the treatment, and the effect lasted for at least 3 months.

It is known that metabolic disorders in apples always cause a decreased starch breakdown. Early theories on cause of bitter pit all were based on starch that remained in the tissue reviewed by Faust and Shear (1968).

The aim of the present work was to study the effect of irradiation on the ultrastructure of plastids and the starch-sugar interconversion in the apple. An identification method of irradiation treatment in apple has also been sought.

Materials and Methods

Raw materials and treatments

Apples (cv. Golden Delicious) were harvested in an orchard of Dánzsентmiklós Horticultural Station near Budapest, Hungary. Harvesting time was 105-110 days following full bloom apples were harvested in Hungary in this stage for winter storage. After picking at the green skinned stage fruits were irradiated by a Co-60 radiation source (0, 0.5, 1, 2 and 5 kGy doses, the dose rate was 1 kGy. Hl) using facilities at the Institute of Isotopes of Hungarian Academy of Sciences. After irradiation, samples were stored for 8 weeks (shelf life at 16°C, 80-90% relative humidity [RH]). This temperature was chosen to hasten the physiological and biochemical changes. The ripening stages of apple and the short time effect (5 days) of irradiation was monitored by the starch iodine pattern index (Reid et al., 1982) (Fig. 1a.). All ultrastructural and chemical investigations were carried out 8 weeks after treatments when the apple skin was yellow and the starch disappeared from the fruit flesh (except 5 kGy) (Fig. 1b).

Fig. 1. Demonstration of starch by starch iodine test in apple (Golden Delicious, stored at 16°C, 80-90% RH) a: 5 days after irradiation; b: 8 weeks after irradiation
The effect of radiation on starch

Electron microscopy and morphometry

Fixation was carried out in 6% (v/v) glutaraldehyde (in 0.035 M K-Na phosphate buffer, pH 7.2) for 2 h at 4°C. After thorough washing in the buffer, samples were postfixed in 1% (w/v) OsO₄ for 1.5 h, dehydrated in an acetone series and embedded in Spurr’s resin. Using flat molds, samples from the skin were oriented for subsequent transverse sectioning.

Sections were cut with a Porter-Blum ultramicrotome equipped with a diamond knife, post-stained with uranyl acetate and lead citrate and examined in a Tesla BS 500 electron microscope operated at 60 kV.

Starch grains were measured with a square lattice grid superimposed on enlarged prints, in 24-27 plastids from the outer hypodermis (h₁–h₃). The volume of starch grains was expressed as a percentage of plastid volume.

Determination of sugar

10 g of the skin or flesh sample was homogenized with 50 ml of 80% ethanol for 5 minutes. Each filtrate was evaporated to dryness to remove ethanol, then redissolved in 100 ml of water. Each solution was analyzed for soluble sugars by high pressure liquid chromatography (HPLC) using a LABOR MIM (Hungary) apparatus combined with a Beckman pump (USA) and RI detector (Beckman), having sensitivity of 2×10⁻⁵. The column was Chromsil-NH₂ (6 μm, 250x4.6 mm), operated at 45°C with
acetonitrile-water (75:25) as mobile phase at 1 ml.min⁻¹ flow rate.

**Determination of starch**

5 g of skin or flesh of apple was homogenized with 100 ml of 1 N HCL for 5 minutes. The homogenate was refluxed on a boiling water bath for 3 hours, made up to 1000 ml, glucose determined by HPLC and expressed as starch.

**Results and Discussion**

The skin of stored apples examined after different radiation doses showed several ultrastructural changes. Data concerning the epidermis and hypodermis will be evaluated separately. Plastids in the epidermis of the control sample were roundish with many electron-translucent vesicles. They generally contained dense inclusions but not starch grains (Fig. 2a). Unevenly distributed starch grains occurred in the epidermis of samples irradiated with 1 kGy or higher doses (Fig. 2b-c). The normal organization of the cell was broken down by an irradiation dose of 5 kGy (Fig. 2d).

Plastids in hypodermal cells of the control sample contained small and large grana. In the outer hypodermis (h₁) plastoglobuli were electron translucent, and starch grains were rare and small. In deeper hypodermal layer (h₂-5, according to Bain and Mercer, 1963) plastoglobuli are more electron dense, and plastids are free of starch (Fig. 3a). In the 0.5 kGy sample the lumina of thylakoids in hypodermal plastids were often electron dense (Fig. 3b). Structures similar to prolamellar bodies could be seen in inner layers of hypodermis (Fig. 3c).

Earlier this structure was considered as a characteristic feature of the etioplasts; later it was established that prolamellar bodies also appear to differentiate following exposure to continuous red light (Boothman et al., 1971) or white light (Rascio et al., 1980). They are formed when the equilibrium of the membrane components (or their synthesis) is disturbed. We suppose, that in our case the prolamellar body-like structure is the result of disturbance of the membrane equilibrium caused by ionizing radiation.

Fig. 3. Ultrastructure of plastids in the hypodermis of samples stored for 8 weeks (Golden Delicious apple) (a: control; b, c: 0.5 kGy), t = thylakoid, g = granum, pb = prolamellar body-like structures. Bars = 1 μm.
The effect of radiation on starch

### Table 1

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Starch content as % of plastid</th>
<th>as % of glucose after hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$0.38 \pm 0.21$</td>
<td>$2.83 \pm 0.92$</td>
</tr>
<tr>
<td>0.5</td>
<td>$1.52 \pm 0.69$</td>
<td>$2.85 \pm 0.91$</td>
</tr>
<tr>
<td>1</td>
<td>$15.82 \pm 2.39$ ***</td>
<td>$6.32 \pm 0.90$ ***</td>
</tr>
<tr>
<td>2</td>
<td>$28.53 \pm 3.16$ ***</td>
<td>$6.56 \pm 0.85$ ***</td>
</tr>
<tr>
<td>5</td>
<td>$38.21 \pm 3.72$ ***</td>
<td>$15.06 \pm 2.40$ ***</td>
</tr>
</tbody>
</table>

$x = \text{average}; \; s = \text{standard deviation};$

**xx** significantly differed from the control, at level 95%.

**xxx** significantly differed from the control, at level 99%.

Plastids of the hypodermis of 1 kGy apple cortex contained small and large starch grains (Fig. 4a) in addition to occasional prolamellar body-like structures. Apples irradiated with 2 kGy contained conspicuous starch grains, mainly in the outer hypodermal cells (Fig. 4b), but cells with damaged membranes also occurred. Large starch grains remained in the outer layer of the hypodermis (h.) of the 5 kGy irradiated apples. In the inner layer chloroplasts with large amounts of starch were observed (Fig. 4c). According to the results of morphometry, the starch content of the hypodermal cells had already differed significantly at an irradiation dose of 1 kGy from the control (Table 1). According to the chemical analysis more starch remained in the skin at a dose 1-5 kGy. The data of morphometry and chemical analysis correlate well.

Fruit flesh was also examined for starch grains. Both in the control and irradiated (0.5-2 kGy) flesh, the small plastids were free of starch (Fig. 5a-b). Starch grains were present in cells of 5 kGy irradiated apple (Fig. 5e). This was also evident from the iodine test (Fig. 1b).

Fig. 4. Ultrastructure of plastids in the hypodermis of samples stored for 8 weeks (Golden Delicious apple) (a: 1 kGy, b: 2 kGy, c: 5 kGy), s = starch. Bars: 1 μm.
The analysis of sugars indicated that glucose concentration increased in the flesh with the increasing radiation doses, which means that most of the decomposed starch was converted into glucose during storage after irradiation (Fig. 6). At the same time in the skin of apple the glucose, fructose and sucrose concentration increased only up to 1 and 2 kGy, then decreased (Fig. 7). Both phenomena have also been observed in sweet potato roots as a function of radiation dose (Hayashi and Kawashima, 1982a, b).

Several factors may have influence on preserving starch in the irradiated fruits. These include Ca mobilization from the flesh to the skin (Kovács et al., 1985; Kovács and Zackel, 1987) and effects involving the integrity of membranes (Romani et al., 1968; Isherwood, 1976). The question is further complicated by the fact that Ca also has an effect on the state of the membranes. Further investigations are needed for understanding of the interaction of these factors.

Fig. 5. Ultrastructure of plastids in the flesh of samples stored for 8 weeks (a: control; b: 0.5 kGy; c: 1 kGy, D: 2 kGy, e: 5 kGy), s = starch. Bars: 1 µm.
The effect of radiation on starch

Fig. 6. The effect of irradiation on the sugar content in the flesh of apple stored for 8 weeks

Acknowledgements

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References


Discussion with Reviewers

T. Hayashi: What is the purpose of irradiating apple? If it is disinfection, the dose required is in the range of 0.1 to 1 kGy, which is lower than that used in your study. Why did you choose the dose range in your study?

Authors: The 0–5 kGy dose range was chosen to follow up the effect of irradiation on starch. The aim of apple irradiation is either disinfection (0.1–1 kGy) or to extend shelf life using irradiation (1 kGy) in combination with Ca-treatment.

Reviewer IV: Could you give some information on the location of the samples with respect to the cross-section of the fruit?

Authors: Samples for both the skin and flesh were collected from the region of the largest diameter, the flesh samples were cut out halfway between the carpels and the skin.

D. Lewis: How many samples did you examine and how did you select the plasids?

Authors: Two fruits were selected from each group, 10–12 samples were cut out of the skin and flesh of each. Each plastid in a micrograph was measured for starch and the micrographs were selected on the basis of their quality.

T. Hayashi: How did the contents of starch and sugars change during storage after irradiation?

Authors: For the first run we did not examine time-dependence of these processes, but it will be really important when developing a method from these results for identifying irradiated apples.

B. Pineran: What is the large dark area in your Figure 2a at the bottom right of the plastid? What are the other rounded structures in this plastid? The latter look like lipid material to me.

Authors: Such large dark areas are called inclusion bodies by several authors, they may be of proteinaceous nature (Ames and Pirourn, 1974: Amer. J. Bot. 61, 794–797; Varkey and Nádakovářen, 1982: New Phytol. 92, 273–279). The other rounded structures are plastoglobuli.

Reviewer IV: In Fig. 6, glucose concentration in flesh at 5 kGy is greatly increased over the control level. In Fig. 1b, the starch level at 5 kGy is also increased. Normally, in starch–sugar interconversion if one component increases there should be a decrease in the other. For both components to increase is anomalous. This point should be addressed.

Authors: On Fig. 6, a significant glucose accumulation is perceptible. Possibly the glucose originating from the starch breakdown cannot be used in the metabolic pathway due to the considerable radiation damage to the cells; while in samples irradiated with smaller doses glucose does not accumulate but it is used up in the increased respiration. (Dharkar and Sreenivasan, 1966: Food Irradiation Proc. of Symp., 6–10 June, Karlsruhe, pp: 635–649; Van Kooy, 1966: Preservation of fruit and vegetables by irradiation, Proc. of Panel, 1–5 August, Vienna, pp. 29–43; Kovács and Vas, 1974: Acta Alimentaria, 3, 19–25).