JOURNAL OF AGRICULTURAL SCIENCE IN FINLAND
Maataloustieteellinen Aikakauskirja
Vol. 58: 27—32, 1986

^{45}Ca mobility and distribution during ripening and maturation of Rutgers and RIN tomatoes.

L.R. HOWARD,[1] A. UUSI-RAUVA and J.J. LAINE[2]

University of Arkansas, Department of Food Science, Route 11, Fayetteville, AR, 72703

University of Helsinki, Faculty of Agriculture and Forestry, Instrument Centre, Viikki, SF-00710 Helsinki, Finland

University of Helsinki, Department of Food Chemistry and Technology SF-00710 Helsinki, Finland

Abstract. Radioisotope ^{45}Ca was used to examine changes in levels of bound and soluble calcium during tomato fruit ripening, and the distribution of calcium in different regions of Rutgers and Rin (non-ripening) tomato tissue. Levels of cell wallmiddle lamella bound ^{45}Ca decreased readily in pericarp tissue during ripening of Rutgers tomatoes with only a small decrease being observed in RIN fruit. No significant increase in soluble ^{45}Ca was observed for either genotype during ripening. Decreasing levels of bound and soluble ^{45}Ca were observed from calyx to blossom end of pericarp tissue in Rutgers and RIN fruits. Low levels of bound ^{45}Ca were found in the inner locular walls at an early stage of tomato ripening. The implication of low levels of cell wall bound calcium in relation to catabolic changes associated with ripening are discussed.

Index words: Ca mobility in tomato tissue. Rutgers and RIN tomatoes.

Introduction

Calcium has been shown to affect many aspects of fruit ripening. In tomatoes, postharvest application of calcium retarded the ripening process of mature green fruit and fruit that had commenced ripening (WILLS and TIRMAZI, 1979). The retardation of the ripening process was attributed to calcium inhibition of mitochondrial and pectic enzymes (WILLS and RIGNEY, 1979). Many studies have implicated the pectic enzyme polygalacturonase (PG) in softening of tomato tissue (PRESSEY, 1979; HOBSON, 1981; HUBER, 1984). Calcium plays a major role in structural integrity of cell walls due to its ability to crosslink pectic substances (GRANT et al., 1973; REES, 1975). Incorporation of calcium into the cell wall results in enhanced tissue resistance to PG, although the ecaxt chemical nature of resistance is unresolved (WILLS and TIRMAZI, 1979; BUESCHER and HOBSON, 1982).

Several studies have reported that cell wall bound calcium declines prior to, or simultaneously with the initiation of ripening

[1] To whom correspondence should be sent.

[2] Present Address: Finnish Meat Research Centre, Box 56, 13101 Hämeenlinna, Finland

(SUWAAN and POOVAIAH, 1978; POOVAIAH, 1979; RIGNEY and WILLS, 1981). RIGNEY and WILLS, (1981) have suggested that mobility of cell wall bound calcium might initiate ripening and precede PG activity which has also been postulated to initiate tomato ripening (NG and TIGCHELAAR, 1977; TIGCHELAAR et al., 1978; POOVAIAH and NUKAYA, 1979). Studies performed on the nonripening, RIN, tomato mutant lend further support to the scheme, since bound calcium levels in RIN increase throughout maturation and little or no PG activity develops (SUWANN and POOVAIAH, 1978; BUESCHER and TIGCHELAAR, 1975).

Recent studies have indicated possible mechanisms of calcium removal from cell walls. BUESCHER and HOBSON (1982) demonstrated that calcium removal by chelators such as EDTA or citrate allowed extensive degradation of tomato cell walls by PG. BRADY et al. (1985) reported that pectic polymers of a firm and soft tomato cultivar were equally susceptible to PG hydrolysis in the presence of citrate which also demonstrates a strong role for calcium in limiting degradation by PG. BRADY et al. (1985) failed to observe a decrease in levels of cell wall bound calcium during ripening, results that conflict with those reported by others (SUWAAN and POOVAIAH, 1978; POOVAIAH, 1979; RIGNEY and WILLS, 1981). Furthermore, RUSHING and HUBER (1985) were unable to induce ripening in mature green and RIN tomatoes by vacuum infiltration of various chelating agents.

Thus, the exact role of calcium in tomato ripening is still unresolved. In this study, we utilized the radioistope ^{45}Ca as a trace in order to follow calcium solubilization and distribution in RIN and Rutgers tomato fruit. Use of radioisotopes in plant nutrition studies is widespread, however, ^{45}Ca has not been previously used to observe changes in cell wall bound Ca during tomato ripening.

Materials and methods

Plant Material

Tomatoes *(Lycopersicon esculentum* Mill) cultivars Rutgers and RIN (isogenic to Rutgers) were grown in sand in a temperature controlled greenhouse (19—23°C), with supplemental lighting. The supplimentary lightning was 250 W/m² by Hg-LX-400 mercury lamps and the period was 16 hours/day. The humidity of the room ranged between 50—70 % during the cultivation period. Plants received a standard nutrient solution daily containing as follows: macronutrients (mEq/1) N 16.2, P 1.8, K 9.9, Ca 8.0, Mg 3.7, 53.8, and micronutrients (ppm): Fe 1.38, Mn 1.38, B 0.28, Zn 0.14, Co 0.14, Mo 0.03. Plants were trained to one stem and fruits were tagged at the time of anthesis. Blossoms were thinned to allow two to three fruits per flower cluster with a maximum of ten fruits per plant. Rutgers fruits were harvested and sorted into the following ripeness classes: mature green (MG), breaker (B), turning (T), pink (p), and red (R) using a U.S.D.A. Visual Aid TM-L-1 (ANON., 1975). Days from anthesis that corresponded to the color stages of Rutgers were used to obtain fruit of the nonripening (RIN) mutant at known stages of development. Stage one fruit corresponding to mature green were harvested 60—63 days after anthesis while stage two fruit corresponding to red ripe were harvested 79—81 days after anthesis.

Sample Preparation and Analysis

Tomato pericarp tissue including the peel was dissected into three equal portions from the distal to proximal end of the fruit. A fourth sample was taken from the locular walls at the interior of the fruit. Dissected samples were immediately freeze dried and stored at —20°C. Representative samples were finely ground to pass through a 60 mesh screen. The samples were analyzed for soluble and bound ^{45}Ca by a modified method of

SUWAAN and POOVAIAH (1978). The tissue was leached with distilled water for 1 hr in a cold room (4°C). Samples were centrifuged at 15,000 g for 30 min and the supernatant filtered through Whatman No. 541 ashless filter paper. Two additional aliquots of distilled water were added to each sample followed by centrifugation and filtration. Filtrate was collected and brought to final 100 ml volume and used to analyze soluble ^{45}Ca. The pellet was ashed at 600°C for 6—7 hours, dissolved in 5 % HCl and used to determine bound ^{45}Ca.

Isotope Application and Procedure

Radioisotope ^{45}Ca, as $^{45}CaCl_2$ (2.1. mCi/ml original activity) was applied at 100 μCi per plant upon flowering of the first clusters of approximately 80 % of the plants.

Samples (1.0 ml) from soluble and bound ^{45}Ca fractions were placed into vials which contained 10 ml of PCS liquid scintillation cocktail (Amersham). Radioactivity was measured with a liquid scintillation counter (LKB Wallac Rackbeta 1215). Activity was expressed as disentigrations per minute (dpm). All samples were corrected for background and radioactive decay.

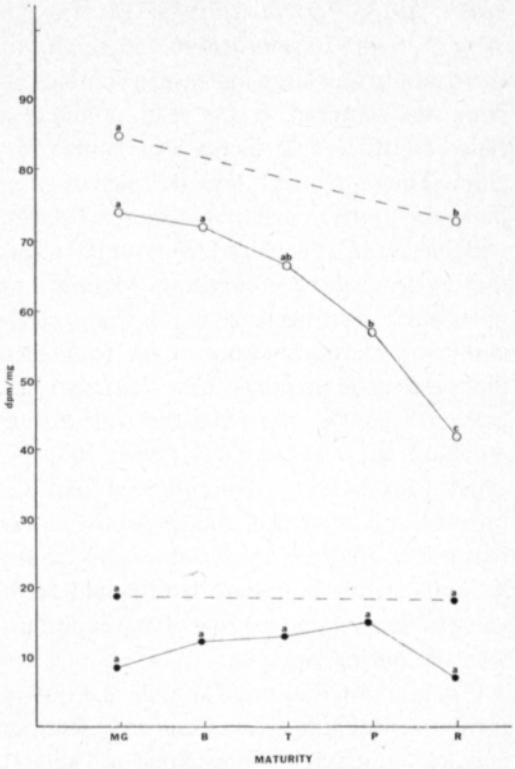

Fig. 1. Levels of bound and soluble ^{45}Ca during ripening of Rutgers and aging of RIN tomato fruits. Broken lines indicate RIN, continuous lines, Rutgers. Open points (○) = bound ^{45}Ca, closed points (•) = soluble ^{45}Ca. Points on each curve followed by the same letter are not significantly different at the 0.05 level (LSD).

Statistical Analysis

Duplicate determinations of three representative fruit from each maturity stage were examined. Five maturity stages were examined from Rutgers and two stages from RIN. Variance analysis with LSD (0.05 level) was used to compare the means.

Results and discussion

Rutgers and RIN tomatoes both contained a large proportion of ^{45}Ca bound to cell wall material (Fig. 1). Assumably the majority of bound ^{45}Ca serves as a structural component of the CW-ML complex. Low levels of soluble ^{45}Ca were observed in both Rutges and RIN tomatoes.

In Rutgers, a significant decrease in bound ^{45}Ca of percarp tissue was observed as fruits ripened from mature green to pink. After the pink stage, bound ^{45}Ca continued to decline. No increase in soluble ^{45}Ca was observed during ripening of Rutgers fruit.

Bound ^{45}Ca in pericarp tissue of RIN fruit decreased from stage one to stage two. These two maturity classifications, based on days from anthesis, correspond to mature green and red Rutgers fruit, respectively. Decreased levels of bound ^{45}Ca were not accompanied by an increase in soluble ^{45}Ca. RIN fruits contained greater levels of bound and soluble ^{45}Ca than Rutgers throughout ripening.

These results support previous observations that bound calcium in Rutgers tomatoes declines during ripening (SUWAAN and POO-

VAIAH, 1978; POOVAIAH, 1979; RIGNEY and WILLS, 1979). In contrast to the results of these authors no large increase in soluble calcium was observed. BRADY et al. (1985) also failed to observe an increase in soluble calcium. They attributed lack of calcium solubilization to the interaction of soluble calcium with intracellular acids, which resulted in formation of insoluble complexes. Possibly a similar mechanism occurred in this study or calcium was translocated out of the fruit. Studies performed on apples have demonstrated calcium translocation from the fruit during moisture stress (WILKINSON, 1968; MARTIN, 1969). MARTIN (1967) demonstrated that ^{45}Ca injected into apple peel and carpel tissue was retranslocated to the nearby leaves and shoots. A similar study in tomato fruit would be of value in determining the role of calcium translocation during ripening.

Calcium distribution of soluble and bound forms according to tissue location in Rutgers and RIN tomatoes is presented in Table 1. Bound calcium in Rutgers fruits was highest at the calyx and lowest at the blossom end for both mature green and red tomatoes. A similar trend for bound calcium was also observed for RIN tomatoes at corresponding maturities. It is apparent that bound calcium is not distributed uniformily within the fruit, presumably due to immobility of the ion in phloem and nonvascular tissue (BANGERTH, 1973, FERGUSON, 1979).

Decreasing levels of soluble ^{45}Ca from the calyx to blossom end of the fruit were also observed in Rutgers and RIN tomatoes. Low levels of calcium at the blossom end of the fruit could play a role in promotion of blossom end rot, either through reduction of cellular cohesion or increased membrane permeability (BANGERTH, 1973, SHEAR, 1975; SIMON, 1978).

Levels of bound ^{45}Ca tended to be very low at the interior of both RIN and Rutgers fruit during ripening. Tomato locular and surrounding tissues are a rich source of organic acids, particularly citric and malic acids (STEVENS et al., 1977). These acids could have a two fold effect on calcium removal from cell walls through: 1) displacement of calcium by ionized protons, 2) chelation of calcium by free carboxyl groups. This is of intrest since incipient ripeness of Rutgers fruits is initiated in the interior tissues. Removal of calcium from cell walls in the interior of matur green Rutgers fruit could possibly trigger the chain of events as envisioned by RIGNEY and WILLS (1981). At the very least this could lead to reduced resistance of the polygalacturonan chain to polygalacturonase. Decreasing levels of bound calcium in the interior and lower regions of Rutgers fruits during ripening corresponds with visual observance of color development during fruint ripening, since ripening commences in the inner locular walls and proceeds upward from blossom to calyx end of the fruit. This trend was not observed in RIN fruit which fail to ripen.

These results demonstrate that a substantial reduction in bound calcium of Rutgers tomatoes occurs during ripening. We have also

Table 1. ^{45}Calcium Distribution and Incorporation Into Soluble and Bound Forms In Rin and Rutgers Tomatoes.

	Rutger (MG)		(R)		RIN (1)[w]		(2)	
Position[x]	Bound	Soluble	Bound	Soluble	Bound	Soluble	Bound	Soluble
1	[y]49.15a[z]	4.30b	25.82a	4.15a	63.43a	9.68a	53.73a	10.52a
2	38.48b	3.98b	22.37a	3.50ab	46.03b	6.97b	38.65b	8.93ab
3	30.06c	2.76b	18.38b	2.10b	32.80c	5.85b	30.07c	7.35b
4	30.52c	6.47a	16.48b	4.36a	28.09c	4.70b	22.28d	9.52a

[w] Stage 1 and 2 Rin fruit correspond to mature green (MG) and red (R) Rutgers fruit according to days after anthesis.

x Positions 1—3 refer to apical, middle, and distal regions of pericarp tissue. Position 4 refers to inner locular walls.

y Values are expressed as dpm/mg.

z Means labeled by the same letter within each column are not significantly different from each other at the 0.05 level (LSD).

demonstrated that a decreasing gradient of bound calcium exists from the calyx and proceeds to the blossom end of both RIN and Rutgers tomatoes. It is suggested that during the early ripening stages, a reduction in bound calcium in the interior and lower portions of Rutgers fruits could severely limit the fruit's resistance to deteriorative changes.

Acknowledgement. The senior author would like to thank the Department Head of Food Chemistry and Technology, Dr. P. Koivistoinen for providing funding and facilities to carry out this work while on leave from the University of Arkansas. This investigation was also supported in part by a research grant from Oy Gustav Paulig Ab, SF-00810 Helsinki, Finland. Sincere appreciation is also extended to Dr. R. W. Buescher for disucssion and revision of the manuscript.

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Ms. received November 19, 1985

^{45}Ca-isotoopin liikkuvuus ja jakautuminen Rutgers ja RIN-tomaattilajikkeissa kypsymisen aikana

L.R. Howard[1], A. Uusi-Rauva, J.J. Laine[2]

University of Arkansas, Department of Food Science, Route 11, Fayetteville, AR, 72703

University of Helsinki, Faculty of Agriculture and Forestry, Instrument Centre, Viikki, SF-00710 Helsinki, Finland

University of Helsinki, Department of Food Chemistry and Technology SF-00710 Helsinki, Finland

Radioaktiivista ^{45}Ca-isotooppia käytettiin tutkittaessa sitoutuneen ja liukoisen kalsiumin määrän muutoksia tomaatissa sen kypsyessä sekä kalsiumin jakautumista kypsymättömän tomaatin eri osissa. Tomaattilajikkeeet olivat Rutgers ja RIN. Soluseinien keskilevyjen sitoma ^{45}Ca-isotoopin määrä pienentyi voimakkaasti perikarppisolukossa Rutgers lajikkeen kypsyessä, kun taas RIN lajikkeella pienentyminen oli vähäistä. Liukoisen ^{45}Ca-isotoopin määrässä ei havaittu merkittävää lisäystä kummankaan lajikkeen kypsymisvaiheessa. Tutkimuksessa havaittiin molemmilla lajikkeilla liukoisen ja sitoutuneen ^{45}Ca-isotoopin määrissä pienentymistä perikarppisolukossa verhiöstä kukkapohjaan. Tomaatin kypsymisen varhaisessa vaiheessa todettiin sitoutuneen ^{45}Ca-isotoopin alhainen määrä sisemmissä vaskulaariseinämissä. Tutkimuksessa selvitettiin lisäksi soluseinään sitoutuneen kalsiumin alhaisen määrän vaikutus katabolisiin muutoksiin kypsymisvaiheessa.

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[1] To whom correspondence should be sent.

[2] Present Address: Finnish Meat Research Centre, Box, 56, 13101 Hämeenlinna, Finland