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SCANNING ELECTRON MICROSCOPY OF CELLULAR STRUCTURE OF GRANNY SMITH AND RED DELICIOUS APPLES

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Abstract

Immature and mature Granny Smith and Red Delicious apples (*Malus domestica* Borkh.) were studied with scanning electron microscopy (SEM). Parenchyma cells were observed to form a net-like pattern in sections transverse to the stem-calyx of the apple. Bundles of about six intact cells were connected, creating large intercellular spaces. The intercellular spaces were different in shape from different perspectives to the cut surfaces; round in stem-calyx transverse sections and elliptical in stem-calyx cross sections. Cell areas, cell lengths and intercellular space areas were determined with image analysis. The patterns of mature apple cell structure of both cultivars were observed to be similar to the patterns of immature apple cell structure. Both cell area and intercellular space area were larger in mature than in immature apples. Cells of mature apples were longer and more elliptical than cells of immature apples. The patterns of intercellular space area and cell length in Granny Smith and Red Delicious apple cultivars were not the same.

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Key Words: Apple cellular structure, scanning electron microscopy, Granny Smith apple, Red Delicious apple, *Malus domestica* Borkh., immature apple, mature apple, cell area, intercellular space area, cell length.

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Introduction

The cell structure of apple flesh was investigated in the early 1940s (Smith, 1940; Tukey and Young, 1942) with optical microscopes. Later, a more developed stereoscopic microscope was applied by Reeve (1953) to observe apple cellular structures.

During the past 20 years, transmission electron microscopy (TEM) has been the primary instrument used to observe apple cell ultrastructure. TEM ultrastructure studies illustrated cell organelles and cell wall changes of apple tissues as maturity increased (Saikia, 1969; Ben-Arie et al., 1979) and physiological development occurred (Mahanty and Fineran, 1975; Fuller, 1976).

In recent years, scanning electron microscopy (SEM) was applied in a few studies of the physical properties of apple cells (Diehl et al., 1979; Simons and Chu, 1980; Bolin and Huxsoll, 1987; Kovács et al., 1988). With the great depth of focus from the SEM, details of cell concavity and cell arrangement of apple tissues can be observed. More information about apple cell structure is provided with SEM than is available from previous investigations of apple cell structure.

Reeve and Leinbach (1953) and Reeve (1970) related cell structure, size of cells, intercellular spaces and composition as factors influencing textural qualities associated with fresh and processed fruits and vegetables. Reeve and Neufeld (1959) proposed that cell size had a definite effect on canned peach texture. Large cell volumes in peach pieces (19.9-21.8 μm^3) resulted in a coarse, stringy and often ragged texture. Small cell volumes in peach pieces (10.2-12.9 μm^3) resulted in a firm, fine and coherent canned peach texture.

Granny Smith and Red Delicious apples were selected cultivars for this research, since both are principal cultivars produced in Washington State.

Immature apples of both cultivars were investigated to provide baseline cell structure and morphology.

The objectives of this research were to characterize cell structure of Granny Smith and Red Delicious apple fruits, and to study cell size, cell shape and intercellular space area of immature and mature apples.

Materials & Methods

Granny Smith and Red Delicious apples (*Malus domestica* Borkh.) were harvested from trees growing in the Washington State University campus orchard. Immature fruits of both cultivars were picked in the last week of August, 1986. Mature Red Delicious apples were harvested on October 6, 1986. Mature Granny Smith apples (1985 apples) were obtained from the Postharvest Physiology Laboratory, Washington State University. Scanning electron microscopy (SEM) examinations of mature apple tissues were from post-climacteric fruits. Mature Red Delicious apple tissues were analyzed one month after harvesting. Mature Granny Smith apple tissues were analyzed after 7 months of controlled atmosphere storage.

Preparation of apple tissues

Fifteen millimeter diameter cylinders were cut through the center of Granny Smith and Red Delicious apples at right angles to the stem-calyx axis with a cork borer. Prior to punching out a cylinder, a downward arrow (stem to calyx) was marked on the apple skin at the end center of the cylinder as an orientation mark. The cylinder was sliced into 2 mm thick discs, from approximately 1 mm under the skin through the core line. Each side of each disc was labelled; the surface toward the skin was labelled A, and the surface toward the core was labelled B. Small 2 x 4 mm pieces of apple tissue were cut from the center of each disc and labelled by cutting the point from one or two corners with a razor blade. One cut corner on the 4 mm side indicated the upward portion (stem) and two cut corners on the 4 mm side indicated the downward portion (calyx) of apple fruit. As labelled apple tissues on stubs were viewed, one cut on the 2 mm side on the left-bottom corner and two cut corners on the right hand side indicated a surface A perspective. Surface B perspective was indicated with the appearance of one cut corner on the 2 mm side on the right-bottom corner and two cut corners on the left hand side.

Preparation for Scanning Electron Microscopy

Marked apple pieces were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 24 h at 4° C. The apple pieces were washed in three changes of 0.1 M cacodylate buffer (pH 7.3) for 10 min each to remove excess glutaraldehyde which will react with osmium tetroxide and leave a dense precipitate on the surfaces of apple tissues (Trump and Ericsson, 1965; Trump and Bulger, 1966). The apple pieces were post-fixed with 2% aqueous osmium tetroxide for 1 h at 4° C. Dehydration was accomplished in an ethyl alcohol series (10%, 20%, 30%, 40%, 50%, 60%, 70% for 10 min each ; 80%, 90% and 95% for 15 min each and 100% 3 times for 15 min each). Ethanol dehydrated apple pieces were critical point dried (Bomar SPC-1500) using carbon dioxide as the transitional fluid. The dried apple pieces were sputter coated (Hummer-Technics) with 300 Å gold and observed with an ETEC U-1 scanning electron microscope (Hayward, CA) operated at 20 kV.

Cell measurement

Cell area, intercellular space area and cell length were measured using a Bioquant II image analysis system (Boyle Instruments, Gig Harbor, WA). Cell area and intercellular space area were measured from SEM micrographs of surface A and/or B of apple discs. The cut corners on apple tissues were observed as diagonal cut areas and used to measure cell length.

Results & Discussion

The gross cell structures of Granny Smith and Red Delicious apples were generally similar. As seen in Fig. 1A-D and 2A-D, parenchyma cells are loosely arranged in a net-like pattern in the outer and middle regions of the apples. Bundles of about six intact cells are attached together to create large intercellular spaces. However, the intercellular space sizes became smaller as the distance from the skin increased toward the core line in stem-calyx transverse sections (Fig. 1A-F, 2A-F). Long elliptical cell shape and an increase in the number of cells in the inner area are related to reduction in size of intercellular spaces. The shapes of intercellular spaces change from round to ellipsoid from different perspectives to the cut surfaces. The stem-calyx transverse section SEM micrographs illustrate round intercellular spaces (Fig. 1A-F, 2A-F, 3A-F, 4A-F). The stem-calyx cross sections SEM micrographs illustrate ellipsoid intercellular spaces (Fig. 5A-F). Observations of apple intercellular space shapes are in agreement with intercellular space shapes previously observed by Reeve (1953) under a stereoscopic microscope.

The cell area from stem-calyx transverse sections became slightly smaller as the distance increased from the skin to the core line (Fig 1A-F, 3A-F). The shape of cells appeared to be

isodiametic within 6 mm beneath the skin. Overall, apple cell shapes tend to become more elliptical and longer toward the core line (Fig. 5A-F).

Cell structure of Granny Smith and Red Delicious apples

Cell area Table 1 illustrates that cell areas of immature Red Delicious apples were larger than cell areas of immature Granny Smith apples. Cell areas of immature Granny Smith apples were smallest in the region 2 - 4 mm above the core line compared to cell areas closer to the skin (Table 1, Fig. 3D,E). However, cell areas were nearly the same size among different regions overall. Cell areas of outer cells near the skin of immature Red Delicious apples (.021 - .024 mm^2) appeared larger than the middle cells (.015 - .018 mm^2) or the cells near the core (.015 - .018 mm^2) (Fig. 4A-F).

Cell areas from all sections of mature Red Delicious apples were larger than cell areas of mature Granny Smith apples (Table 1). Cells throughout mature Granny Smith apples were nearly the same size. Cells approximately 3 mm beneath the skin of mature Red Delicious apples were observed to be the largest in size compared to cells in the rest of Red Delicious apples.

In general, the outer cells of immature and mature apples of both cultivars were slightly larger than cells in the middle or inner regions (Table 1). The observations of consistently larger cells near the skin were in contrast to previous observations that cells towards the skin were slightly smaller than cells located farther away from the skin in apple cultivars examined by Reeve (1953).

In both cultivars, cells of immature fruits were smaller in area than cells of mature fruits (Table 1). Increase in cell size is pronounced in mature Red Delicious apple cells compared to immature Red Delicious apple cells. The ratio of mature:immature cell area of Red Delicious apples illustrated that cells in the middle region of mature apples are approximately two times larger in size than cells in the middle region of immature Red Delicious apples.

Overall, the cell areas of Granny Smith apples were smaller than the cell areas of Red Delicious apples in both immature and mature apples.

Intercellular space area Immature Granny Smith and Red Delicious apples contained a similar percentage of intercellular space area (ISA), ranging from 11 to 32% in an SEM micrograph area of 3.04 mm^2. The percentage ISA in the outer regions (21-32%) of Granny Smith and Red Delicious apples was greater than the percentage ISA in the middle regions (11-17%) or the percentage ISA in the inner regions (11-15%) of both cultivars.

Table 1. Cell area of immature and mature Granny Smith(GS) and Red Delicious(RD) apples.

cultivar	sample region	cell area[a] (mm^2) immature	mature	ma:im ratio
GS	outer	.017<1A>	.022<1A>	1.3
		.019<1B>	.020<1B>	1.1
	middle	.019<2B>	.019<3B>	1.0
		.012<4B>	.017<4B>	1.4
	inner	.015<5B>	.019<7B>	1.3
		.020<6B>	.018<8B>	0.9
RD	outer	.024<1A>	.027<1A>	1.2
		.021<1B>	.042<1B>	2.0
	middle	.018<2B>	.033<3B>	1.8
		.015<3B>	.033<4B>	2.2
	inner	.018<4B>	.027<7B>	1.5
		.015<5B>	.022<8B>	1.5

a) means of 17-40 cells
<> slice no. and surface of perspective

Table 2. Intercellular space areas(ISA) of immature and mature fruits of Granny Smith(GS) and Red Delicious(RD) apples.

region	ISA[a](%) GS immature	GS mature	RD immature	RD mature
outer	27.25	43.23	26.51	30.39
middle	12.18	27.88	15.71	35.97
inner	13.06	28.02	12.24	13.64

a) calculated from

$$\frac{\text{total intercellular space area}(mm^2)}{3.04\ mm^2\ \text{SEM picture area}} \times 100$$

Table 3. Cell length of immature(im) and mature(ma) Granny Smith(GS) and Red Delicious(RD) apples.

cultivar	region	slice No. im	ma	cell length[a] im	ma	ma:im ratio
GS	outer	1	1	0.227	0.250	1.1
		2	2	0.186	0.248	1.3
	middle	3	4	0.208	0.278	1.3
		4	5	0.203	0.304	1.5
	inner	5	7	0.196	0.340	1.7
		6	8	0.233	0.442	1.9
RD	outer	1	1	0.237	0.327	1.4
		2	2	0.201	0.334	1.7
	middle	3	5	0.197	0.287	1.5
	inner	4	7	0.266	0.340	1.3
		5	8	0.201	0.354	1.8

a) means of 25 cells, measured in mm

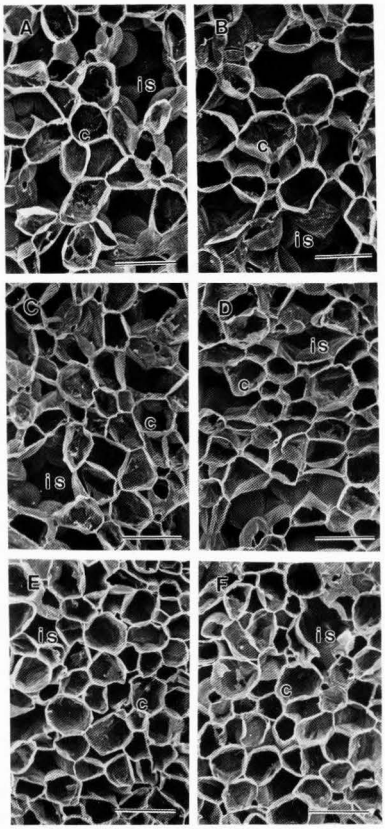

Fig. 1 Scanning electron micrographs showing cells (c) and intercellular spaces (is) of mature Granny Smith apples. A and B illustrate outer region, C and D illustrate middle region, E and F illustrate inner region. Bar = 250 μm.

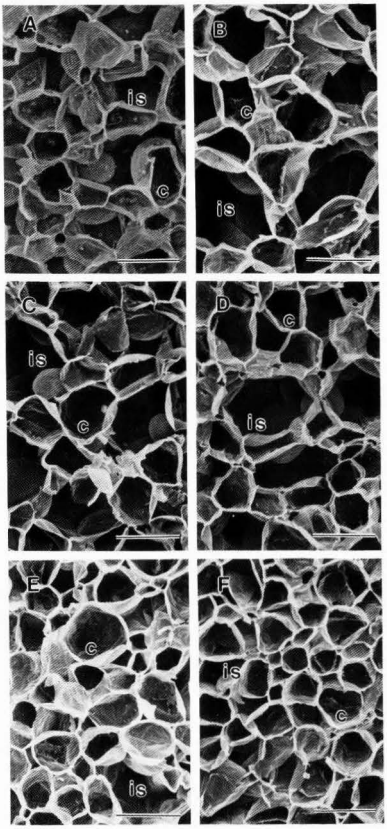

Fig. 2 Scanning electron micrographs showing cells (c) and intercellular spaces (is) of mature Red Delicious apples. A and B illustrate outer region, C and D illustrate middle region, E and F illustrate inner region. Bar = 250 μm.

However, no differences were noted among percentages of intercellular space areas between cultivars or regions of the immature apples.

Mature Granny Smith apples exhibited an intercellular space pattern different from mature Red Delicious apples. The ISA from outer and inner regions of Granny Smith apples were larger than the ISA from outer and inner regions of Red Delicious apples (Table 2). In Granny Smith apples, the ISA in the middle (27.88%) and inner (28.02%) regions were fairly similar, but the ISA in the outer region (43.23%) was larger (Table 2). In Red Delicious apples, the ISA from the outer region (30.39%) was similar to the ISA from the middle region (35.97%), which were larger than the ISA from the inner region (13.64%) (Table 2).

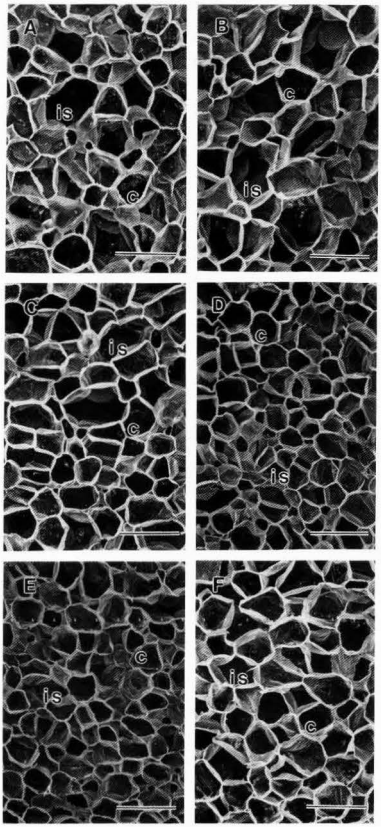

Fig. 3 Scanning electron micrographs showing cells (c) and intercellular spaces (is) of immature Granny Smith apples. A and B illustrate outer region, C and D illustrate middle region, E and F illustrate inner region. Bar = 250 μm.

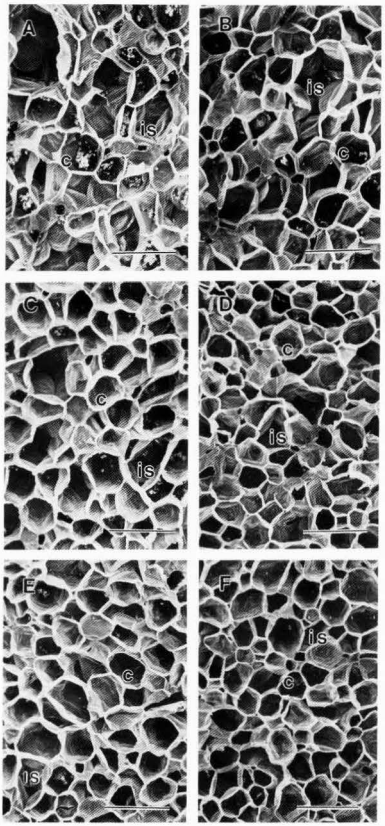

Fig. 4 Scanning electron micrographs showing cells (c) and intercellular spaces (is) of immature Red Delicious apples. A and B illustrate outer region, C and D illustrate middle region, E and F illustrate inner region. Bar = 250 μm.

The intercellular space patterns of mature Granny Smith and Red Delicious apples followed the same pattern as immature apples (Table 2). The percentage intercellular space of Granny Smith apples increased with increasing maturity in the outer, middle and inner areas observed (Table 2). Tukey and Young (1942) proposed that intercellular spaces increase greatly during the two months preceding fruit maturity. However, only the intercellular spaces in the middle region of mature Red Delicious apples were larger than the intercellular spaces in the middle region of immature Red Delicious apples (Table 2).

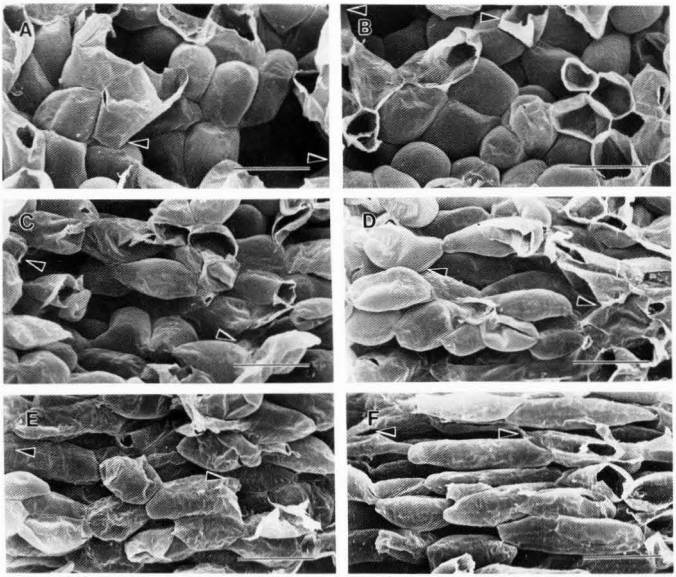

Fig. 5 Scanning electron micrographs showing cell shape changed from skin to the core of mature Granny Smith apples. A and B illustrate outer region, C and D illustrate middle region, E and F illustrate inner region. Bar = 250 μm. Arrows indicate intercellular boarders.

Cell length Cell length in the inner region of immature Granny Smith and Red Delicious apples exhibited the longest cells compared to cell length in other regions (Table 3). Cells in the outer region of immature Red Delicious apples were longer than cells in the outer region of immature Granny Smith apples (Table 3).

The cells became significantly longer as both Granny Smith and Red Delicious apple fruits aged, except in the 3 mm portion of Granny Smith apples near the skin (Table 3). Granny Smith apples exhibited a different cell pattern than Red Delicious apples. Granny Smith apple cells tended to increase in length, 0.250 to 0.442 mm, as distance of cells increased from the skin to the core line. Red Delicious apple cells were longest in the region under the skin and above the core line and shortest in the middle region of the apple.

The patterns of cell area, cell length and percentage intercellular space of mature Granny Smith and Red Delicious apples were similar to the patterns of cell area, cell length and intercellular

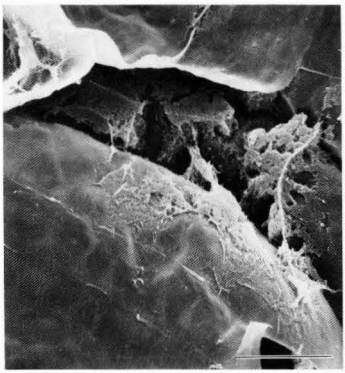

Fig. 6 Scanning electron micrograph showing apparent solids leakage in intercellular spaces of Granny Smith inner area. Bar = 50 μm.

space of immature Granny Smith and Red Delicious apples. The cell areas and intercellular spaces became larger and cell lengths became greater as maturation increased. Mature Red Delicious apple cells were larger and longer than Granny Smith apple cells.

MacArthur and Wetmore (1941) reported that cell differentiation of apple fruits was completed by the end of June. Three weeks were necessary to complete cell differentiation in McIntosh after full bloom (Tukey and Young, 1942). Immature apples of both cultivars in this study were in the enlargement period of fruit growth. Enlargement of fruit results primarily from an increase in intercellular spaces, cell lengths and/or cell sizes. Yamaki et al.(1979) proposed that the total polysaccharide content of Japanese pear cell wall per cell (DNA content basis) increased rapidly during the pre-enlargement period and remained almost constant during the enlargement period, while the pear fruits enlarged dramatically. The accumulation of polysaccharide substances in the cell walls during the pre-enlargement was necessary for increasing cell size and length.

Granny Smith apple tissues increased significantly in intercellular space area and cell length, but not in cell area during maturation. Red Delicious apple tissues increased significantly in cell length and cell area, but not in intercellular space area during maturation. Theoretically, cell walls of immature apples will be thicker than cell walls of mature apples, since immature apple cells have less surface area than mature apple cells with almost constant total polysaccharide content (Yamaki et al., 1979). Davis and Gordon (1980) reported that cell walls of carrot phloem in early developmental stage were thick with fibrous material. The cell walls of phloem in fully mature carrots were thin and firm with no fibrous material. Cell wall thickness was not measured in this research, however, cell walls of mature apples (Fig 1,2) appeared thicker than immature apple cell walls (Fig 3,4).

Solids leakage into intercellular spaces was observed in the inner region of mature Granny Smith apples (Fig. 6). Saikia (1969) observed transmission electron micrographs with accumulated solids in apple intercellular spaces during the climacteric increase in respiration rate. The accumulation of solids in intercellular spaces suggested that changes in cell membrane structure occurs without changes in cell morphology.

Conclusions

Characterization of the tissue and cell structure of Granny Smith and Red Delicious apples was achieved through use of scanning electron microscopy and image analysis. General cell arrangement and morphology of Granny Smith and Red Delicious apples remained the same during maturation while cell area, intercellular space area and cell length increased. Changes of cell shape and size suggest that the angle of observation for apple tissue must be precise to avoid misinterpretation of cell changes.

Acknowledgements

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References

Ben-Arie R, Kistev N, Frenkel C. (1979). Ultrastructural changes in the cell walls of ripening apple and pear fruit. Plant Physiol. 64, 197-202.

Bolin HR, Huxsoll CC. (1987). Scanning electron microscope/ image analyzer determination of dimensional post-harvest changes in fruit cells. J. Food Sci. 52, 1649-1650, 1698.

Davis EA, Gordon J. (1980). Structural studies of carrots by SEM. Scanning Electron Microscopy. 1980; III: 601-611.

Diehl KC, Hamann DD, Whitfield JK. (1979). Structural failure in selected raw fruits and vegetables. J. Texture Stud. 10, 371-400.

Fuller MM. (1976). The ultrastructure of the outer tissues of cold-stored apple fruits of high and low calcium content in relation to cell breakdown. Am. Appl. Biol. 83, 299-304.

Kovács E, Keresztes A, Kovács J. (1988). The effects of gamma irradiation and calcium treatment on the ultrastructure of apples and pears. Food Microstructure 7, 1-14.

MacArthur M, Wetmore RH. (1941). Development studies of the apple fruit in the varieties McIntosh Red and Wagener. II. An analysis of development. Can. J. Bot. 19, 371.

Mahanty HK, Fineran BA. (1975). The effects of calcium on the ultrastructure of Cox's Orange apples with reference to Bitter Pit disorder. Aust. J. Bot. 23, 55-65.

Reeve RM, Leinbach LR. (1953). Histological investigations of texture in apples. I. Composition and influence of heat on structure. Food Res. 18, 592-603.

Reeve RM. (1953). Histological investigations of texture in apples. II. Structure and intercellular spaces. Food Res. 18, 604-617.

Reeve RM, Neufeld CHH. (1959). Observation on the histology and texture of Elberta peaches from trees of high and low levels of nitrogen nutrition. Food Res. 24, 552-563.

Reeve RM. (1970). Relations of histological structure to texture of fresh and processed fruits and vegetables. J. Texture Stud. 1, 247-284.

Saikia BN. (1969). A study of ultrastructural changes during maturation and ripening of the apple fruit. Ph. D. thesis, Washington State University, Pullman, WA. pp. 179.

Simons RK, Chu MC. (1980). Scanning electron microscopy and electron microprobe studies of bitter pit in apples. In: Atkinson D, Jackson JE, Sharples RO, Waller WM. (eds). Mineral Nutrition of Fruit Trees. Butterworths, London, pp. 57-69.

Smith WH. (1940). The histological structure of the flesh of the apple in relation to growth and senescence. J. Pom. Hort. Sci. 18, 249.

Trump BF, Bulger RE. (1966). New ultrastructural characteristics of cells fixed in a glutaraldehyde-osmium tetroxide mixture. Lab. Invest. 15, 368.

Trump BF, Ericsson JLE. (1965). The effect of the fixative solution on the ultrastructure of cells and tissues. A comparative analysis with particular attention to the proximal convoluted tubule of the rat kidney. Lab. Invest. 14, 1245.

Tukey HB, Young JC. (1942). Gross morphology and histology of developing fruit of the apple. Botanical Gazette 104, 4-25.

Yamaki S, Machida Y, Kakiuchi N. (1979). Changes in cell wall polysaccharides and monosaccharides during development and ripening of Japanese pear fruit. Plant Cell Physiol. 20, 311-321.

Discussion with Reviewers

K. G. Lapsley: Do you feel you have examined enough apple tissue to be able to account for the variability in structure within any one apple?
Authors: More apples should be examined. However, the selected immature and mature apples were similar in size, shape and weight. Therefore, we believe the results are representative of the cell structure of Red Delicious and Granny Smith apples.

E. Kovács: How was the degree of ripening determined?
Authors: Apples were harvested after quality tests on color, acidity, pH, soluble solids, starch content and firmness. The respiration rates of apples were examined to predict ripening stage or maturity.

E. Kovács: What sampling method did you use?
Authors: Apples were selected by size, shape and appearance. After the first selections, apples of similar weight and density were selected to represent Granny Smith and Red Delicious cultivars.

E. Kovács: Is there any correlation between the textural changes and the size of cells/ size of intercellular spaces? Have you data on it?
Authors: Yes, fruits consisting of large cells with considerable intercellular space give a coarse or spongy texture. In contrast, fruits consisting of small cells with little intercellular space give a smooth texture (Reeve, 1970). We did not examine the relationship of cell size or intercellular space size to apple texture in this research.

E. Kovács: The quality of fruits are influenced by the weather, nutrition and humidity conditions of soil. How do these factors influence the size of cells and intercellular spaces?
Authors: Cell size, cell number and intercellular space of apples are affected by several factors (Westwood et al., 1970; Proc. Am. Soc. Hort. Sci. 91:51-62). Under adequate soil moisture and excess N fertilizer, apple cells tend to increase in size. The amount of intercellular space is related to fruit size, the larger the fruits the greater amount of intercellular space. Soil with available nutrients and adequate moisture available for growth is expected to provide large fruit from light-cropping trees.

E. Kovács: Do you plan to investigate processed apple products in the same way?
Authors: No, we have no plan to do further research on processed apple products.

K. G. Lapsley: Is 3 dimensional mapping of an apple possible yet?
Authors: Three-dimensional mapping is possible for parenchyma cells in the middle area of an apple perpendicular through the stem-calyx axis. Cell structure mapping at the stem or calyx ends must be based on representative observations of specific areas to complete 3-dimensional mapping of an entire apple.

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Technical Note: ENCAPSULATION OF VISCOUS FOODS IN AGAR GEL TUBES FOR ELECTRON MICROSCOPY

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Abstract

Viscous food is aspirated into a glass capillary tube with the inner diameter of approximately 0.5 mm if the food is to be examined by transmission electron microscopy. If the sample is destined for examination by scanning electron microscopy, it is aspirated into a Pasteur pipette having the diameter of 1.0 mm. In each case, the lower end of the glass tube is sealed with a droplet of 40°C warm 3% agar sol. After the sol solidifies, the pipette is dipped in the same agar sol and a coating, 0.2 to 0.5 mm thick, is formed around the glass tube by manipulating it while the sol is still liquid. Dipping may be repeated in order to form a uniform coating of desired thickness. The agar gel sleeve is then trimmed, and the pipette is withdrawn, whereby the sample slides into the agar gel sleeve. The free upper end of the agar gel tube is then sealed with a drop of the agar sol. The subsequent preparation of the encapsulated sample for electron microscopy is the same as that of a solid sample.

KEY WORDS: Agar gel tubes, Electron microscopy, Encapsulation, Viscous foods.

Introduction

Encapsulation of biological samples in agar gel tubes for subsequent examination by electron microscopy has been described by several authors [1, 3-7]. This technique may be used for scanning electron microscopy (SEM) as well as transmission electron microscopy (TEM). Food samples as diverse as milk [3, 4], orange juice [5], stirred yoghurt [1], and mayonnaise [2] have successfully been examined using this technique.

The techniques developed earlier consist of forming an agar gel tube around a piece of steel wire or a glass rod and using the wire or the glass rod as a piston when aspirating the sample into the agar gel sleeve. In this note, an easier and a more rapid approach is described. Its main features were published earlier [6].

Materials and Methods

Agar sol (3%) was made using distilled water and was stirred continually with a magnetic bar at 40°C.

A glass Pasteur pipette with inner diameter of 1.0 mm was used as obtained from the supplier for samples destined for SEM or was drawn out into a capillary tube to an inner diameter of approximately 0.5 mm for use with samples destined for TEM.

Commercial stirred-style yoghurt samples were aspirated into the thin capillary tubes to a length of approximately 2 mm, or were aspirated into the Pasteur pipettes to a length of 15 to 20 mm. The lower ends of the glass tubes were wiped clean with paper tissue and were sealed with droplets of the agar sol (Fig. 1).

After the sealed end had solidified, the capillary tube or the Pasteur pipette was dipped into the agar sol and then was manipulated to form a thin layer of agar gel on the glass surface around the sample. Dipping was repeated once or twice to form a uniform agar gel layer around each sample. The agar gel sleeve was then trimmed at the upper end of the sample and removed. The capillary tube or the pipette was then withdrawn from the agar gel sleeve, whereby the sample slid from the glass tube into the gel tube. The sample column in the agar gel tube was somewhat shorter than was its initial length in the glass tube following its removal because the inner diameter of the agar gel tube was larger than that of the glass tube. The freed upper end of the agar gel tube was then trimmed with a blade approximately 0.5 mm above the sample and was sealed with a droplet of the agar sol. It was then possible to handle the encapsulated samples as solid samples during the subsequent preparatory steps for electron microscopy.

Results and Discussion

The advantages of encapsulating viscous samples for electron microscopy have already been discussed in the literature [1-5]. However, some of the techniques are quite laborious. A high degree of manual dexterity is required to properly aspirate the sample into the agar gel tube using it as a cylinder while the solid rod around which the tube had been formed is used as a piston. This procedure leads to another problem, that is the need to seal both ends of the tube after the sample is aspirated and the piston is withdrawn.

The suggested technique (Fig. 1) simplifies the encapsulation procedure and markedly increases the productivity of the technician. The manipulation required to form a uniform gel coating by dipping the glass tube

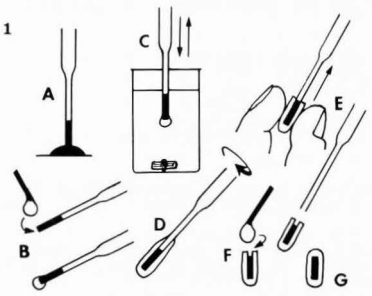

Fig. 1. Encapsulation of viscous food samples in agar gel tubes. A: Aspirate sample, B: Seal lower end of tube, C: Dip into agar sol, D: Rotate tube to form agar gel sleeve, E: Withdraw glass tube, F: Seal upper end of tube, G: Encapsulated sample.

containing the sample into agar sol can easily be learned. Should this appear difficult even after making several attempts, gels may be cast around the glass tubes using a technique that has been described elsewhere [5].

Very viscous samples such as Cream cheese can be encapsulated also provided that the above technique is slightly modified. As it is impossible to aspirate them into the tubes, the samples are placed in the tubes by repeatedly tapping the tubes into the samples which are placed on a firm support such as a microscope glass slide.

The ease with which the sample may be transferred from the glass tube into the agar gel sleeve depends on the viscosity of the sample and also on the quality of the seal at the lower end of the tube. If difficulties are encountered and the agar gel sleeve collapses, the seal should be strengthened with another agar sol droplet and a thicker agar gel sleeve should be formed.

Resin blocks containing food samples which had initially been encapsulated in agar gel tubes must be trimmed in such a way that the entire agar seal is removed along with the bordering area where the food sample and the agar gel may be mixed together.

Food samples encapsulated in agar gel tubes for subsequent SEM examination may be freeze-fractured following their fixation, dehydration, and impregnation with absolute ethanol. The agar gel coating may be left on the sample [1] or may be removed (Fig. 2).

Foods, which disintegrate in aqueous solutions and yet cannot be placed in glass capillaries to be prepared for TEM, may be coated with a thin agar gel layer in a different way. The sample is placed on the tip of a needle and touched with a droplet of warm agar sol (Fig. 3). The sol coats the food particle and immediately solidifies. The bead thus formed is removed from the needle and the exposed area of the food sample is sealed with another agar sol droplet. Again, the encapsulated sample can be treated as a conventional solid particle in any further preparatory steps.

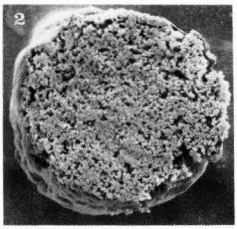

Fig. 2. SEM of stirred yoghurt. The sample was encapsulated in an agar gel tube, fixed, dehydrated, and freeze-fractured. The agar gel tube was removed prior to SEM examination.

Fig. 3. Coating of thick food samples with agar gel for TEM if the sample is too thick to be aspirated into a glass capillary tube.

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References

1. Allan-Wojtas P, Kaláb M. (1984). A simple procedure for the preparation of stirred yoghurt for scanning electron microscopy. Food Microstruc. 3, 197-198.
2. Dylewski DP, Unger RS, Martin RW. (1988). Microstructure of oil-in-water fat spreads. Food Microstruc. 7 (in press).
3. Henstra S, Schmidt DG. (1970). Ultrathin sections of milk by means of the microcapsulation method. (In German). Naturwissenschaften 39, 247.
4. Henstra S, Schmidt DG. (1974). The microcapsule technique. An embedding procedure for the study of suspensions and emulsions. LKB Application Note No. 150.
5. Jewell GG. (1981). The microstructure of orange juice. Scanning Electron Microsc. 1981;III: 593-598.
6. Kaláb M. (1987). Encapsulation of viscous suspensions in agar gel tubes for electron microscopy. Electron Microsc. Soc. Am. Bull. 17(1), 88-89.
7. Salyaev RK. (1968). A method of fixation and embedding of liquid and fragile materials in agar microcapsulae. Proc. 4th Europ. Reg. Conf. Electron Microsc. Rome, II, 37-38.

Discussion with Reviewers

D. P. Dylewski: I would like to express some caution for the application of the above procedure to samples high in their lipid content. I would like to discuss the following three points:
(1) How is a sample, especially one high in lipid content, affected morphologically when it is dipped repeatedly into molten agar at a temperature of 40° to 45°C?
(2) When the removal of the space occupied by the glass tube is completed, how does the sample "pack" into the agar cylinder? Would mayonnaise, for example, retain its native or near natural form, that is, tight packing of lipid droplets?
(3) Using Salyaev's method [7], the sample is drawn in one step into the agar cylinder. Using your method, the sample is handled twice: first it is drawn into the pipette, then blown out into the agar gel cylinder. What are the effects, if any?

Author: The method has been developed in order to facilitate SEM investigation of small hard particles causing grittiness in protein-based milk products such as stirred yoghurt and soft cream cheese. The effect of temperature on high-fat foods such as mayonnaise would have to be tested. If problems are encountered, the agar gel tube around the glass capillary tube may be formed by smearing the agar sol around it rather than dipping the glass tube into the agar sol. This would limit the effect of heat on the sample as the smear cools quite rapidly.

Your concern for the loss of space and the effects of handling are closely related. In my opinion, greater effects on the packing of the sample constituents may be anticipated to originate from the initial aspiration rather than from the subsequent sliding of the sample into the agar gel tube. In viscous samples, there is no continuous matrix that would be at risk of disintegration. The distribution of corpuscular components would not be affected unless air is aspirated along with the samples and a new gas-liquid interface is thus formed in them.

It is advisable, however, that the effects of the factors which you have mentioned be investigated in the case of viscous high-fat foods.

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