Secretory Cavity Development and Its Relationship with the Accumulation of Essential Oil in Fruits of *Citrus medica* L. var. *sarcodactylis* (Noot.) Swingle

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**Abstract**

The developmental types of secretory cavities in *Citrus* remain controversial. The relationship between secretory cavity development and the accumulation of essential oil in fruits of *Citrus* species is also unknown. In order to develop better insights into these problems, histological, histochemical, and cytochemical methods were used to investigate secretory cavity development and the accumulation of essential oil at different developmental stages of fruits of *Citrus medica* L. var. *sarcodactylis* (Noot.) Swingle. The results indicate that the secretory cavity of the variety seemed to originate from an epidermal cell and a subepidermal cell. These two cells underwent successive divisions, resulting in the formation of two parts: (i) a conical cap; and (ii) a globular gland. The formation of the lumen was schizolysigenous. Regular changes in the size of vacuoles and the accumulation of essential oil were revealed during the process of secretory cavity development. In addition, when fruits were a light yellow or golden color, the structure of secretory cavities was well developed and the content of essential oil in a single fruit reached a maximum. It would be most appropriate to collect the fruit as a medicinal material at this time.

**Key words:** *Citrus medica* var. *sarcodactylis*; development; essential oil; fruit; secretory cavity.


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*Citrus medica* L. var. *sarcodactylis* (Noot.) Swingle is a species of Rutaceae, the fruit of which is used as a traditional Chinese medicinal material for the treatment of asthma and stomach ache (Pharmacopoeia Commission of People’s Republic of China 2000) owing to its essential oil. The essential oil is also an important additive in foods and cosmetics because of its elegant perfume (Jin and Du 2002). Secretory cavities are not only the common structure in Rutaceae, but also the primary sites of the synthesis, secretion, and collection of essential oil (Esau 1965). With respect to the developmental types of secretory cavities in *Citrus*, different viewpoints have been expressed by many investigators. According to Haberlandt (1914), secretory cavities develop schizolysigenously, whereas others (Fohn 1935; Heinrich 1966, 1969) supported the view of a lysigenous process. Recently, Turner et al. (1998) considered that the central space of the secretory cavities was formed schizogenously. However, Bennici and Tani (2004) seem to support the view of Haberlandt (1914). These authors believe that cavity formation and enlargement occur by schizogenous and lysigenous processes that overlap one another (Bennici and Tani 2004).

Differences regarding the developmental type of secretory cavities in *Citrus* remain. It is of significance, in theory, to determine the morphological essence of the secretory cavities in *Citrus* through further investigation of the developmental type of secretory cavities and fruit development in *C. medica* var. *sarcodactylis*. In addition, secretory cavities develop along with the growth of the fruit. During the development of secretory cavities in *Citrus*, different viewpoints have been expressed by many investigators. According to Haberlandt (1914), secretory cavities develop schizolysigenously, whereas others (Fohn 1935; Heinrich 1966, 1969) supported the view of a lysigenous process. Recently, Turner et al. (1998) considered that the central space of the secretory cavities was formed schizogenously. However, Bennici and Tani (2004) seem to support the view of Haberlandt (1914). These authors believe that cavity formation and enlargement occur by schizogenous and lysigenous processes that overlap one another (Bennici and Tani 2004).

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cavities, the essential oil content shows regular changes. Therefore, in the present study, we investigated secretory cavity development in fruits of *C. medica* var. *sarcodactylis*, as well as the accumulation of essential oil during the process of its development, so as to provide morphological and phytochemical evidence for the right time to harvest fruits as medicinal materials.

**Results**

**Histological and cytological traits in the process of secretory cavity development**

Many protuberant bright spots at the surface of the fruits could be seen by the naked eye. In fact, these bright spots were the outer appearance of the secretory cavities and they were usually distributed in different-sized circles (diameter 100–400 µm, mainly approximately 300 µm) and at different depths in the cross-sections of a fruit. In addition, the pericarp included several parenchymal cell layers internal to the circle of secretory cavities. There were obviously intercellular spaces between the parenchymal cells and vascular bundles in the parenchyma.

According to the histological and cytological traits in the process of secretory cavity development, the ontogeny of the secretory cavities could be divided into four stages, as described below.

**Initial cell stage**

The initial cells of the secretory cavity originated from an epidermal cell and a subepidermal cell occurring in the ovary of *C. medica* var. *sarcodactylis*. The two cells had a dense cytoplasm and a large nucleus (Figure 1). They did not divide simultaneously and, generally, the subepidermal cell divided first in a periclinal direction to form two cells (Figure 2); following that, each of the cells divided in a transverse or longitudinal direction to produce four cells (Figure 2). At this point, the epidermal cell started to divide (Figure 3). Through asymmetric divisions in series, several cells of different sizes were formed and were arranged in a pattern resembling a brick wall. Meanwhile, the subepidermal cells continued to divide to form a multicellular group (approximately 10 cells), which appeared oval or round in shape (Figures 3, 4). Both groups of dividing cells together constituted the initial cell group of the secretory cavity.

**Intercellular space-forming stage**

The initial cell group increased in size by continuous cell division. The developing glandular structure appeared to consist of two distinguishable parts: a globular part (main gland) and a conical one, which sat on the former like a cap. The cells in the cap part were small and became mature at once. They connected the main gland with the epidermis and the epicarp was usually concave at this place (Figure 4). Gland cells had a dense cytoplasm, small vacuoles, and a large nucleus with prominent central nucleoli. This meant that they would undergo frequent divisions (Figure 5). At this time, the cell walls among the central cells of the gland cells became partly swollen (Figure 5). On development of the secretory cavity, the swollen cell walls gradually broke and formed an intercellular space.

**Figures 1–9.** Different developmental stages of secretory cavities in fruits of *Citrus medica* var. *sarcodactylis*.

(1–4, 7–9) Light microscopy.
(5,6) Electron microscopy.
(1–4) Initial stage.
(1) An epidermal cell and a subepidermal cell (arrows).
(2) The anticlinal division in an epidermal cell (arrow) and periclinal or longitudinal division in a subepidermal cell forming four cells (arrowheads).
(3) The asymmetric divisions in epidermal cells (arrow) and subepidermal cells becoming a multicellular group (arrowhead).
(4) The secretory cavities consisting of two parts: a global gland; and a conical cap.
(5–7) Intercellular space-forming stage.
(5) The partly swollen cell walls among center cells, showing osmiophilic droplets (arrows) and cell plate (white arrow) in initial cells.
(6) Osmiophilic droplets (arrows) in the cytoplasm and bulbous pockets, osmiophilic vesicles (white arrows) in the cytoplasm.
(7) The formation of the lumen.
(8,9) Lumen-expanding stage.
(8) Separation of the radial walls of epithelial cells (arrowhead).
(9) Tangential prolongation of epithelial cells, containing growing vacuoles (arrows).

Bars, 8 µm (1,2,5); 10 µm (3,7); 13 µm (4); 5 µm (6); 18 µm (8); 15 µm (9). Bp, bulbous pocket; C, cap part; E, epithelial cell; L, lumen; Sh, sheath cell.
surrounded by three to four cells (Figure 6). Soon after that, bulbous pockets with light staining appeared in the cell walls near the intercellular space and some osmiophilic materials were present in the bulbous pockets (Figure 6). The intercellular space may continue to expand along the bulbous pockets, resulting in the formation of a small lumen surrounded by four to five cells (Figure 7). There were three to four layers of cells, big and isodiametric, surrounding the lumen. Of them, some would later become epithelial cells, whereas the others would dissolve and disappear. In addition, there were three to four cell layers consisting of relatively small and flattened cells external to the future epithelial cell layers, which would later turn into sheath cells.

**Lumen-expanding stage**

First, in the future epithelial cell layers, some outer layer cells penetrated between the radial walls of the inner layer cells and became a part of the inner layer of the future epithelial cells, directly surrounding the intercellular space. In this pattern, the number of inner layer epithelial cells increased gradually (Figure 8). Then, all future epithelial cells gradually became vacuolized and underwent tangential prolongation so that the intercellular space increased rapidly (Figures 9, 10). Afterwards, epithelial cell walls became thin and separation of the cell walls occurred. This process was accompanied by strong structural disorganization of the cytoplasm, loss of nuclei, and plasmolysis (Figures 11, 12). An interesting phenomenon was that each of the epithelial cells had a big central vacuole when some of epithelial cells were separated from the neighboring cells and began to dissolve (Figures 11–14). The processes of cell dissolution progressed in a centrifugal direction (Figures 12–14).

On the verge of the mature stage, a remaining damaged cell was observed in the lumen and a few oil materials were released into the lumen as the cell was digested (Figure 15). Finally, only two to three layers of epithelial cells remained to surround an expanding lumen. Through a series of developmental changes, the lumen distinctly expanded and secretory cavities were nearly mature.

**Mature stage**

When the diameter of the lumen reached approximately 300–400 µm, secretory cavities became mature. Mature secretory cavities consisted of two parts: (i) a conical cap; and (ii) a globular gland. At this stage, the lumen was surrounded by one to two cell layers of tangentially elongated and intact epithelial cells in the globular part, which only had small vacuoles and dense cytoplasm. Outside of the epithelial cells, there were five to eight layers of sheath cells with a highly tangentially elongated shape and higher vacuolization than the former cells (Figures 16, 17).

**Histochemical changes of essential oil in the different developmental stages of secretory cavities**

Secretory cavities were the primary sites of accumulation and secretion of essential oil. Essential oil, when stained with Sudan III or Sudan IV, became a red droplet under light microscopy. Essential oil could be detected by transmission electron microscopy (TEM) because it could form an electronically opaque, dark, osmiophilic droplet or a gray osmiophilic vesicle after fixation in osmium tetroxide.

Under light microscopy, red droplets in the initial cells were

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**Figures 10–17.** Different developmental stages of secretory cavities in fruits of *Citrus medica var. sarcodactylis*

(10,11,13–16) Electron microscopy.

(12,17) Light microscopy.

(10–15) Lumen-expanding stage.

(10) Osmiophilic droplets (arrows) and osmiophilic vesicles (white arrows) in the cytoplasm.

(11) Osmiophilic droplets (arrows) and osmiophilic vesicles (white arrows) in the cytoplasm, epithelial cell walls becoming thinner (arrowheads).

(12) Damaged epithelial cells with an abnormal shape and a central vacuole (arrows).

(13) Osmiophilic droplets (arrow) and osmiophilic vesicles (white arrows) were present in the cytoplasm and epithelial cell walls were partly damaged (arrowheads).

(14) Relic of dissolved epithelial cell walls (arrowheads) and dissolving cells (arrows).

(15) Part of a damaged epithelial cell (arrowhead) was still present in the lumen and most epithelial cells were found to be intact surrounding the lumen. In addition, oil materials are being released from a damaged cell and are accumulating in the lumen.

(16,17) Mature stage.

(16) Osmiophilic droplets (arrows) in groups and osmiophilic vesicles (white arrow) in the cytoplasm of epithelial and sheath cells; around the lumen, some extrusive vesicles are filling with osmiophilic material (arrowheads).

(17) Mature secretory cavity.

Bars, 5 µm (10, 14–16); 11 µm (11); 15 µm (12); 13 µm (13); 20 µm (17). C, cap part; E, epithelial cell; L, lumen; Sh, sheath cell.
not observed at the initial stage. At the intercellular space-forming stage, epithelial cells were found to be filled with red droplets (3–4 µm diameter), but there were no red droplets in sheath cells and the parenchyma (Figure 18). Along with the expansion of the lumen, red droplets increased gradually in number and size and most were distributed in epithelial cells (Figure 19). Some red droplets (6–8 µm diameter) were secreted into the lumen (Figure 20). At the mature stage, the lumen of the secretory cavity was filled with large red droplets (10–15 µm diameter), but in different color degree resulting from different density of oils (Figure 21).

Under the electron microscope, a small number of osmiophilic droplets (0.5–1.0 µm diameter) were found to distribute in the initial cells, but osmiophilic vesicles had not yet occurred (Figure 5). At the intracellular space-forming stage, dark osmiophilic droplets (1.0–2.5 µm diameter) appeared in the cytoplasm, as well as in the bulbous pockets. A few gray osmiophilic vesicles (2–4 µm diameter) were also present in the cytoplasm (Figure 6). With the expansion of the lumen, epithelial cells and sheath cells had many osmiophilic vesicles (3–5 µm) and numerous dark osmiophilic droplets existed within the osmiophilic vesicles (Figure 10). While dissolution of epithelial cells occurred, the osmiophilic vesicles appeared more dense in the dissolving cells (Figures 11, 13). Oil materials were released from the damaged epithelial cells and accumulated in the lumen, along with the dissolving epithelial cells (Figures 14, 15). At the mature stage, osmiophilic droplets, aggregated in groups, were distributed in epithelial cells and sheath cells. Osmiophilic vesicles (3–7 µm diameter) were abundant in epithelial cells. In addition, a specific change occurred in the cell walls of epithelial cells close to the lumen. The cell walls close to the lumen looked very dark and some extrusive vesicles containing osmiophilic materials often occurred along these cell walls (Figure 16).

The histochemical technique using Sudan stains was useful for gross staining of essential oil, while also causing fusion of the droplets into large masses (Jayme and Harders-Steinhäuser 1967). In addition, studies have reported that glutaraldehyde fixation followed by osmium tetroxide preserves droplets in a shape most closely similar to that of living cells (Witztum and Zamski 1969). This seems to explain why the diameters of the droplets of essential oil, as observed by Sudan staining and osmium tetroxide and reported above, were different at the same developmental stage of the secretory cavities.

Changes in the essential oil in fruits at different developmental stages

In young fruit, all secretory cavities were almost at the initial stage. The content of essential oil was lowest because essential oil had just started to accumulate in the initial cells. The essential oil content would not increase rapidly until the formation of the lumen. According to differences in color, fruits could be divided into five stages (stages 1–5): dark green, light green, light yellow, golden and yellow brown, respectively (Figure 22). The characteristics of the fruits at different stages are given in Table 1.

All fruits at different stages were sliced up and insolated. Through analysis of the rate of production of essential oil, the rate of production of essential oil was found to be highest at stage 1. During the following stages, the rate of production declined rapidly in stage 2, then increased promptly in stage 3, and finally decreased slowly in stages 4 through to 5. The change in the rate of production of essential oil exhibited an “S” shape (Figure 23).

The rate of production of essential oil changed obviously at different stages of development, as did the content of essential oil in a single fruit with increasing fruit weight. In Figure 24, it can be seen that the content of essential oil in a single fruit increased promptly from stage 1 to stage 3. The content of essential oil in a single fruit reached a maximum in stage 3, which was 284% greater than the content at stage 1. Then, the
essential oil content dropped slowly. A 27% decrease in essential oil content was observed from stage 3 to stage 5.

**Discussion**

**Schizolysigenous development of secretory cavities in fruits of *C. medica* var. *sarcodactylis***

With respect to the developmental type of secretory cavities in *Citrus*, different results have been reported in previous publications. In the early 20th century, based on results obtained with the paraffin section technique, Haberlandt (1914) considered that the formation of the lumen in *Citrus* was schizolysigenous. However, Heinrich (1966, 1969) studied the ultrastructural development of the lumen in *Citrus* and reported that lumen formation was lysigenous in the latter half of that century. Thomson et al. (1976) demonstrated that the secretory cavities of *Citrus sinensis* originated schizogenously at an early stage. Those authors did not exclude the possibility that lysigeny could occur during later stages of cavity development because some damaged cells were observed at this time.

Owing to disputes regarding the type of developmental of secretory cavities in *Citrus*, many researchers have used different methods and techniques to confirm the reliability of the experiment results. Hu and Yu (1993) compared preservation of FAA-fixed cavities embedded in paraffin and resin. They found that epithelial cells were poorly preserved with paraffin embedding, whereas with resin embedding, epithelial cells remained intact. In addition, after comparing several methods, Turner et al. (1998) and Turner (1999) suggested that lysigeny could be a false category of gland development, representing misinterpretation of artefacts. Recently, Bennici and Tani (2004) indicated that the formation of the lumen in *Citrus sinensis* and *C. limon* occurred partly schizogenously and partly lysigenously. These events are not completely separated, but overlap partially. In the present study, our observations revealed that the secretory cavities of *C. medica* var. *sarcodactylis* originated schizogenously at an early stage of cavity development; only dissolution of the middle lamella was observed. However, some epithelial cells began to dissolve and, soon after that, epithelial cells prolongated tangentially to expand the lumen. The dissolving process included strong structural disorganization of the cytoplasm, loss of nuclei, and plasmolysis in cells. Finally, when the dissolving process was completely finished, mature secretory cavities with intact and smooth epithelial cells were formed again. Thus, we believe that the formation of the lumen in fruits of *C. medica* var. *sarcodactylis* is schizolysigenous (schizogenous and lysigenous in turn). (In fact, using the

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**Table 1.** Characteristics of fruits of *Citrus medica* var. *sarcodactylis* at different stages of development (see text for details)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Color</th>
<th>Diameter (cm)</th>
<th>Length (cm)</th>
<th>Weight (g)</th>
<th>Diameter of lumen (µm)</th>
<th>Density of cavities (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dark green</td>
<td>3.5 ± 0.7</td>
<td>6.7 ± 1.7</td>
<td>44.6 ± 18.2</td>
<td>35.6 ± 7.3</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>Light green</td>
<td>6.2 ± 0.7</td>
<td>9.9 ± 4.1</td>
<td>210.4 ± 73.9</td>
<td>135.3 ± 24.5</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>Light yellow</td>
<td>7.7 ± 0.7</td>
<td>11.5 ± 0.7</td>
<td>314.7 ± 47.7</td>
<td>304.2 ± 32.1</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>4</td>
<td>Golden</td>
<td>8.5 ± 0.4</td>
<td>11.9 ± 0.6</td>
<td>377.7 ± 60.3</td>
<td>320.6 ± 30.2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>5</td>
<td>Yellow brown</td>
<td>7.4 ± 0.4</td>
<td>10.2 ± 5.2</td>
<td>286.4 ± 48.1</td>
<td>288.1 ± 48.5</td>
<td>9 ± 1</td>
</tr>
</tbody>
</table>

*Average of the maximum diameter of the lumen (20 samples per stage).

*The number of cavities in a circular area with a diameter of 3 mm in the epicarp (20 samples per stage).
cytochemical localization method, pectinase and cellulase have been shown to participate in different developmental stages of the secretory cavities in *C. medica* var. *sarcodactylis* fruit; SJ Liang et al., unpubl. obs., 2006). These results are different from the situation in most textbooks, in which the glands in fruits of *Citrus* species are reported as examples of lysigenous cavities (Esau 1977; Fahn 1979). The type of developmental of secretory cavities in fruits of *Citrus* is likely to be commonly schizolyssigenous.

In addition, the vacuoles in gland cells changed along with the process of secretory cavity development. At the intercellular space-forming stage, vacuoles were small and dispersed. Along with expansion of the lumen, the vacuolization of epithelial cells increased. When epithelial cells were damaged, the vacuoles were at their maximal size (a large central vacuole). After the mature stage, the central vacule of epithelial cells disappeared and only small vacuoles were observed. We could not determine whether vacuolization of epithelial cells governed the dissolution of the epithelial cells but, in any case, vacuoles at a maximal size (forming a large central vacuole) could be used as a morphological criterion to judge the beginning of the dissolution of epithelial cells. A similar interpretation was considered by Thomson et al. (1976). With further expansion of the lumen, the epithelial cells became vacuolated and fragile, and there were indications of wall degradation suggestive of lysigeny of the cells at this stage (Thomson et al. 1976).

The secretory cavity in fruits of *C. medica* var. *sarcodactylis* seemed to originate from an epidermal cell and a subepidermal cell. These two cells underwent successive division, resulting in the formation of a conical cap and a globular gland. Bosabalidis and Tsekos (1982a, 1982b) gave a similar interpretation for *C. deliciosa*, whereas Fohn (1935) concluded that secretory cavities in *Citrus* developed from groups of meristematic cells located under the protoderm. Only one globular part made up the mature secretory cavity. It is the inconsiderate nature of the cap part in fruits that may explain why the majority of previous studies failed to mention the presence of a cap structure in the secretory cavity of *Citrus* fruit (Knight et al. 2001). If a transection does not pass through this cap part, it is possible to get the wrong impression that secretory cavities are initiated in the parenchyma (Bosabalidis and Tsekos 1982a, 1982b; Knight et al. 2001).

**Characteristics of fruits and the structure of secretory cavities can be used to judge the right time to harvest fruits**

Secretory cavities are not only a typical structure in Rutaceae, but also the primary site of synthesis, secretion, and collection of essential oil (Esau 1965). With the development of fruits, essential oil accumulates continuously in the lumen of secretory cavities. When the fruits mature, the essential oil content decreases as a result of the elimination of the oil. In theory, the fruits should be collected when the essential oil content is at its peak but, in practice, this is difficult. Usually different sized and colored fruits are collected at the same time by medicinal farmers, resulting in a reduction in the quality of the medicinal materials and in the curative effect. Therefore, it is very important to determine a simple and practicable guide for the right time to harvest on the basis of the characteristics and secretory cavity structure in the fruits of *C. medica* var. *sarcodactylis*.

In the present study, we demonstrated that the rate of production of essential oil and the density of secretory cavities were both at a maximum in “stage 1”. However, at this time, the size and weight of the fruits are at their lowest compared with other developmental stages. Observed under TEM, only a small quantity of osmiophilic droplets appeared in epithelial cells (Figure 5). The content of essential oils in a single fruit was at its lowest at this stage too. Even though fruits grew quickly during stage 2, only a few osmiophilic vesicles appeared in the epithelial cells in which the essential oil centralized (Figures 6, 18). In fact, the essential oils content in a single fruit was still low at stage 2. The main time for the accumulation of essential oil was at stage 3, even though fruits grew slowly at this stage. Transmission electron microscopy revealed there was a considerable amount of osmiophilic material in the epithelial and sheath cells at this stage (Figures 11, 13, 14). In addition, numerous essential oils were found to be secreted into and accumulated in the lumen (Figures 19, 20). Thus, the rate of production of essential oil was high and the content of essential oil in a single fruit reached a maximum value. At stage 4, fruits and secretory cavities became mature (Figures 16, 21). Because at this stage the elimination of essential oil started, the essential oil content in a single fruit decreased, but it was only a little bit lower than that at stage 3. At stage 5, the fruits started shrinking and the essential oil content in a single fruit declined markedly. Thus, we have come to the conclusion that stages 3 and 4 (light yellow and golden color; fruit approximately 8 cm in diameter, with a length of approximately 12 cm and weight approximately 400 g) are the right time at which to harvest the fruit. However, there is some disagreement about the right time to harvest fruit; the Pharmacopoeia Commission of People’s Republic of China (2000) recommends that the fruit be harvested when it is light green or light yellow. Moreover, the characteristics of the fruit are easily influenced by the environmental factors. Thus, the time to harvest should be determined on the basis of more stable parameters. Fortunately, the accumulation of essential oil coincided with changes in the diameter of the lumen. When the lumen was circinal in shape and approximately 300 μm in diameter, the epithelial cells were flat and smooth and the essential oils content was at its peak.
Coincidently, the color of the fruits was light yellow and golden. Therefore, the size, shape and structure of secretory cavities could be used as another criterion to determine the right harvest time of fruits.

Materials and Methods

Plant materials

The fruits of *Citrus medica* L. var. *sarcodactylis* (Noot.) Swingle were collected from bergamot trees planted in a cultivation base in Gaoyao County, Guangdong Province, China. Some trees were transplanted to the medicinal botanical garden of the South China Agricultural University. All materials were identified by Bing-Tao Li from the College of Forestry, South China Agricultural University.

Determination of essential oil content

According to the color and size (but mainly the color), fruits were divided into five stages: dark green, light green, light yellow, golden and yellow brown. All fruits were washed, sliced, and then dried by insolation. Using the weight lost on drying (Pharmacopoeia Commission of People’s Republic of China 2000) to determine water content, when the water content was below 10%, dried fruit slices were stored in sealed polyethylene film bags for the following experiments. First, 50 g dried fruit slices from different stages were put into a flask (distilling device) with 500 mL distilled water. The flask was heated and kept boiling mildly for 5 h. After cooling, the essential oil content was measured (Pharmacopoeia Commission of People’s Republic of China 2000).

Frozen sections for light microscopy

Fresh fruits from different stages were cut to a thickness of 40–60 µm using a Leica UCT microtome and then stained with uranyl acetate and lead citrate. Sections were examined and photographed using a Leica (Eindhoven, Netherlands) FEI-TECHNAI 12 TEM.

Thin sections for light microscopy

Fresh fruits from different stages were cut into blocks (1 mm³) and fixed in 5% (v/v) glutaraldehyde in 0.1 mol/L sodium phosphate buffer (pH 7.2) for 4 h at 4 °C, then washed in three changes of the same buffer, post-fixed in 1% (w/v) aqueous osmium tetroxide for 2 h at room temperature, and rinsed in three changes of double-distilled water. Specimens were then dehydrated in a graded ethanol series and embedded in Epon812 (SPI Supplies Division of Structure Probe Inc., West Chester, USA). Specimens were cut to a thickness of 1–2 µm with glass knives on a Leica UCT ultra-microtome. Sections were stained with periodic acid-Schiff (PAS) and Toluidine Blue O (Xu and Hu 1986), examined, and photographed using a Leica DMLB light microscope.

Ultra-thin sections for transmission electron microscopy

The procedure for the preparation of ultra-thin sections for transmission electron microscopy was as described in Thin sections for light microscopy for the first few steps. After being embedded in Epon812, specimens were cut to a thickness of 70–90 nm with a diamond knife on a Leica UCT ultramicrotome and then stained with uranyl acetate and lead citrate. Sections were examined and photographed using a Phillips (Eindhoven, Netherlands) FEI-TECHNAI 12 TEM.

References


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