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Anatomy of Lenticels and The Occurrence of Their Discoloration in Mangoes (*Mangifera indica* cv. Namdokmai)

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ABSTRACT

Lenticels of mango fruit sometimes show discoloration during maturation or after harvest. To investigate the causes of the discoloration of lenticels, microscopical observation of cv. Namdokmai has been carried out. It was clarified that a lenticel developed under each stoma in epidermis. Following the development of fruit lenticel, a hole or opening in it became larger. Cuticular layer covered the surface of fruit except the part of the opening of lenticel. Histochemical staining of discolored lenticel showed the presence of polyphenols as a part of components of discolred cells. Pathological factors were considered not to be the main cause of discoloration of lenticel.

Key words : mango, lenticel, *Mangifera indica*, postharvest, anatomy

INTRODUCTION

Mango (*Mangifera indica*) is one of the popular and typical tropical fruits. The discoloration of lenticels in mango is the serious problem in postharvest of fruits because dark colored dots on fruits first shows the undesirable impression and second is concerned with fruit quality if pathological factors are related with them.

Pantastico (1975) represented the diagram of the mango fruit structure including lenticel. It was however, not the detailed structure. There are almost no literatures which represented the detailed anatomy of lenticels in mango. Moreover, no investigation of the discoloration of mango lenticels was done from physiological and/or biochemical points of view.

In this report, therefore, the investigation to clarify the cause of discoloration of lenticels in mango fruits was tried mainly from anatomical and histochemical view point. If the cause of lenticel discoloration is clarified, effective treatment of mango fruits to prevent the discoloration of lenticels is carried out during maturation or postharvest. For this purpose the structure of lenticel has first been observed. Based on the anatomical features the process of discoloration of lenticels has been investigated.

In this study cv. Namdokmai which is one of the typical varieties in Thailand especially from the view point of export and which shows discoloration of lenticel during maturation has mainly been studied. Another cv. Falan whose lenticels protruding from the surface show no discoloration has also been investigated for comparison.

Concerning terminology of lenticel in fruits, Pantastico (1975) and Fahn (1982) supported the concept that lenticels present on fruits in addition to stems and roots. Esau (1964), however, stated that "lenticel is specially formed in the periderm distinguished from phellem in having intercellular space" and "periderms is secondary protective tissue derived from the phellogen and replacing the epidermis". Fruits show generally no secondary thickening. Strictly speaking, therefore, it is better to use the term "lenticel-like structure" in fruits instead of lenticel. In this report, however, the term lenticel was used because the structure and physiological features of lenticel is considered to be similar to real lenticel.

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MATERIALS AND METHODS

1. Materials

Two cultivars, Namdokmai and Falan, were used for experiments. In Namdokmai sample fruits were collected following the process of fruits development. A description of samples is shown in Table 1.

2. Methods

Fresh sample blocks were fixed with 3% glutaraldehyde immediately after collection. For histochemical observation, 20 μm thick sections were cut by disposable knife using freezing microtome (Lipshaw, Type 1500-N). Sections were stained with Sudan IV, Nile blue, safranin and light green, cotton blue, or Nitroso reaction (Reeve, 1951).

For the detailed observation of lenticel structure, thin sections, 1-2 μm in thickness, were cut by glass or histodiamond knife from Epoxy embedded blocks in LKB Nova ultramicrotome. Thin sections were stained with 0.1% toluidine blue 0 or 0.1% safranin.

The procedure of Epoxy embedding was based on the conventional method. That is, fixed blocks were washed with phosphate buffer (pH 6.98), dehydrated with a series of alcohols, replaced with butylglycidyl-ether and embedded in Epon 812.

For the histochemical staining of sections, tefron plate with small shallow holes was used to protect the small and fragile sections from break.

RESULTS AND DISCUSSION

1. Anatomy of lenticels

It is very important to know the exact structure of lenticels for the consideration of the cause of their discoloration. Figures 1 and 2 show the transverse sections of a lenticel in mature discolored lenticel. The epidermis of mango fruit was covered with cuticular layer. It was especially clear in the sections stained with Sudan IV or Nile blue (Fig. 1). At the part of lenticel, however, small hole presented and cuticular layer showed discontinuity. The cells constituting leticel had smaller diameter than the cells of another part and many intercellular speaces existed (Fig. 2). The cells near hole, moreover, showed sometimes deformation (Fig. 2). To know the anatomy of lenticel, it is important to investigate lenticels not only in mature stage but also in young stage.

In younger fruit, one month after full bloom, many small whity dots were observed by naked eyes or by a magnifying glass. These dots were considered to be the early structure of lenticels judging from their density on the surface. The structure of these whity dots, therefore, were observed.

430 serial sections, 1.5 μm in thickness and 1 μm in width, were observed. From this series of sections the structure which corresponded to a small whity dot became clear (Fig. 3). In one month fruit epidermal cells generally showed regular arangement covered with cuticular layer. The pattern just after cell division was sometimes observed in epidermis and cortex near epidermis. In the epidermis, however, special structure was observed (Fig. 3).

This structure had following features. That is, the cells protruded from the surface, the cover of cuticular layer thinner on this structure, one pair of special epidermal cells existed, the parenchyma cells constituting this structure showed the irregular arrangement and intercellular spaces developed around this structure. From these anatomical features this structure is considered to be the early stage of a lenticel.

The detailed structure of one pair of epidermal cells was observed. These two cells were different from another epidermal cells in their shape and cell contents. The length of this special cell was estimated from serial sections. The length was estimated as 70 μm. This length corresponded more than two times of the length of another epidermal cells. From the observation of serial sections, moreover, it was clarified that the width of pore between two special cells changed and showed the largest width at the central part of the cells (Fig. 3). The cuticular layer which covered epidermis showed, moreover, ledges at the outermost part (Fig. 3). These structural features strongly resembled to a stoma (Fahn, 1982; Esau,

Table 1 Description of sample fruits.

Cultivation variety	Developing stage	The date of collection
Namdokmai	1 month[1]	Jan. 9, 1989
	2 months	Jan. 9, 1989
	3 moths	Jan. 9, 1989
	Mature	Jan. 9, 1989, May 22, 1988
Falan	Mature	Jan. 9, 1989

1 after full bloom

Figure 1 A transverse section of a lenticel stained with Nile blue. Arrows indicate cuticular layer.

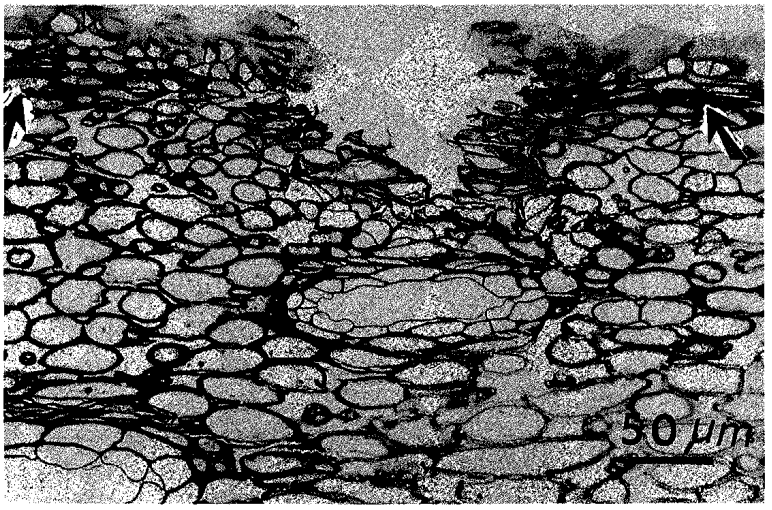

Figure 2 A transverse section of a lenticel stained with toluidine blue 0 (thin section). Arrows indicate thick walled cells.

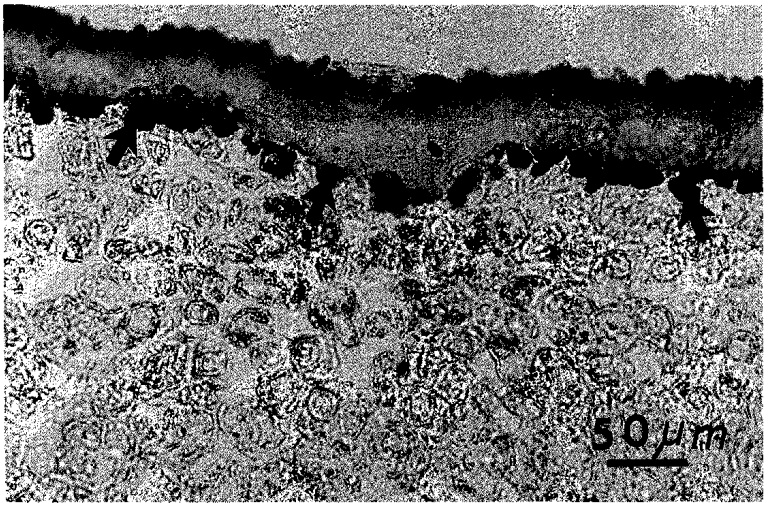

Figure 4 A transverse section of 1 month fruit stained with Sudan IV showing lipid droplets (arrows) near cuticular layer.

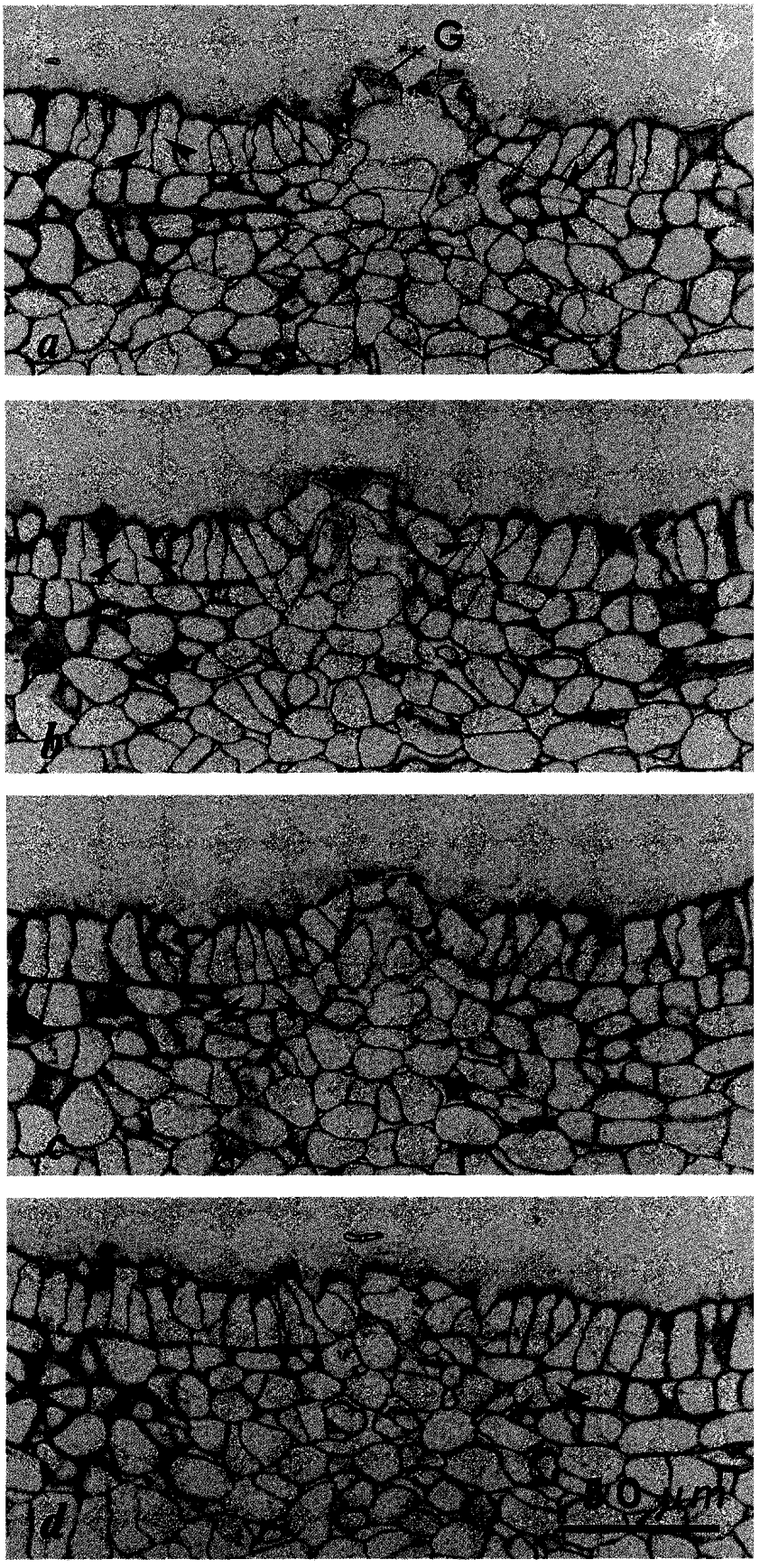

Figure 3 Four transverse thin sections from a serial sections of a lenticel (toluidine blue 0 staining) which show the structural changes from the central part (a) to the peripheral part (d) of a lenticel. Arrow heads show the cells just after cell division. G indicates a guard cell.

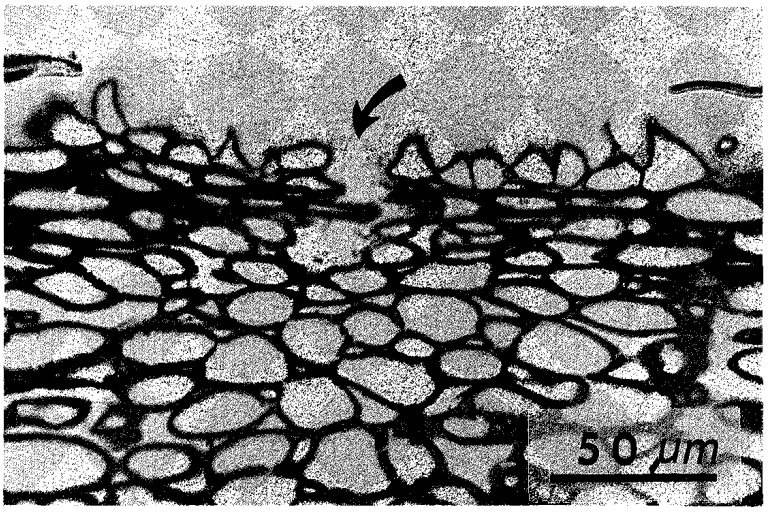

Figure 5 A transverse thin section stained with toluidine blue 0 showing a lenticel (arrow) in 3 months fruit.

Figure 6 A transverse section of a lenticel of Falan double stained with safranin and light green. Arrows indicate the cells with regular arrangement.

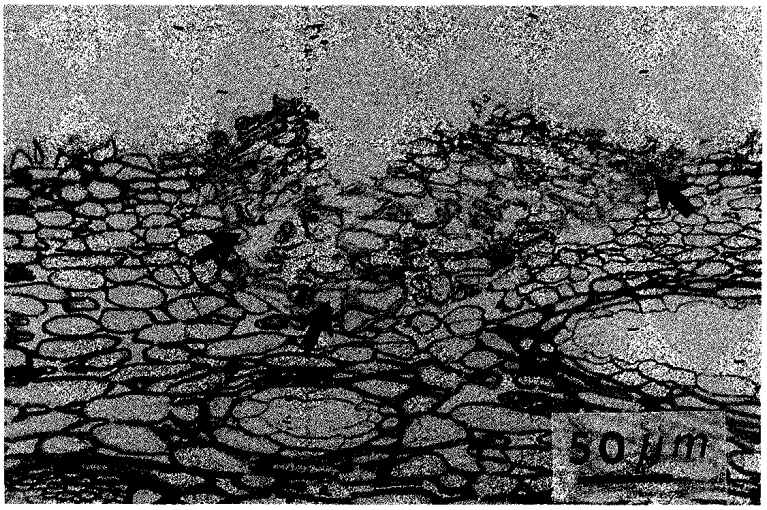

Figure 7 A transverse section of a lenticel of Falan stained with toluidine blue 0 (thin section). Arrows indicate the cells with thick wall.

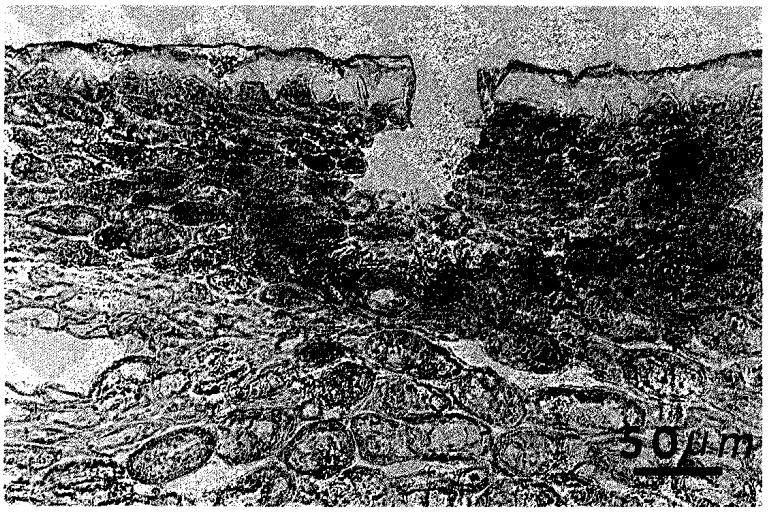

Figure 8 Unstained section showing the color of discolored lenticel in mature Namdokmai.

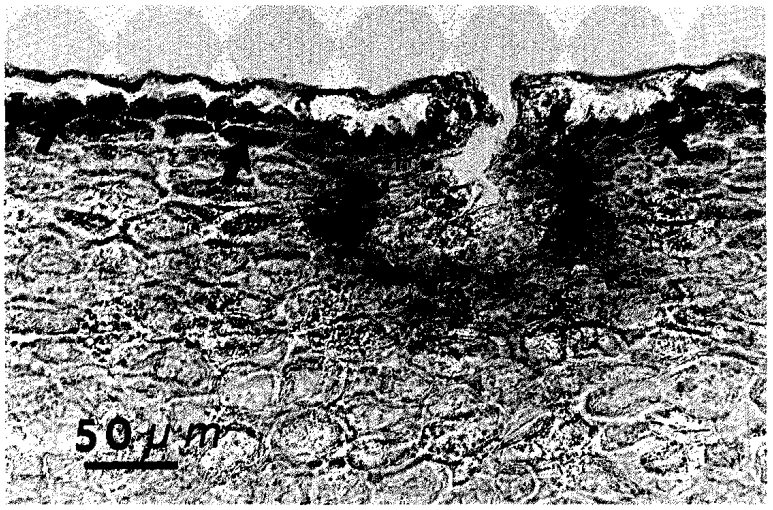

Figure 9 Nitroso reaction of discolored lenticel. Reddish brown indicates the presence of polyphenols. Arrows show the cell with polyphenols containing vacuoles.

Figure 10 A lenticel double stained with safranin and light green showing the necrosis of cells (arrows) constituting lenticel.

1965). The special one pair of cells was, therefore, considered to be a part of a stoma and each cell was considered to correspond to a guard cell.

Concerning the development of lenticel, Fahn (1982) reported that a lenticel develops under each stoma, or under a group of stomata or develop between stomata if the stomata are spacely distributed. The lenticel of mango, at least in Namdokmai, belongs to the category of the former groups.

Many lipid droplets were observed near the cuticular layer in one month fruit (Fig. 4). These lipid droplets were supposed to be related with the formation of culticular layer.

The transverse section of a lenticel in 3 months after full bloom are shown in Fig. 5. The lenticel certainly had small hole. The size, however, is still small. During two month from 1 to 3 months after full bloom mango fruit conspicouosly developed in volume and surface area. Focused on epidermis, for example, it included no cells under division and each epidermal cell became almost largest size in 3 months (Fig. 5). The cell arrangement, moreover, changed to irregular pattern (Fig. 5).

In the discolored lenticel the hole became conspicuously large. The deformation and degradation of cells were also observed near the hole (Fig. 2). In the cuticular layer the folding of it was observed. The hole of lenticel in Fig. 2, moreover, is not the artifact during cutting process because the block was embedded in Epoxy resin. The real hole, therefore, exists in the lenticel.

The conspicuous changes occurred in lenticel structure after 3 months stage of fruit development. To consider the cause of the changes, the cell structure of lenticel was compared with cells which constituted the part among lenticels or background. Firstly, the size of cells constituting lenticel was small. Secondly, many intercellular spaces existed in lenticel. This was considered because of the insufficient increase of cell size in lenticel during the development of fruit. The cell walls became thicker in the tissue between lenticels (Fig. 2) but were rather thin in the cells of lenticel.

From these structural features it was considered that the lenticel was weak point in terms of mechanical properties. Although the real trigger which caused the conspicuous chagne in lenticel is still unknown, lenticels are considered to be sensitive and weak for some factors from outside.

2. Comparison of lenticel structure between cultivars

Among many cultivars of mango, some varieties show discoloration of lenticels and others do not. To consider the differences among cultivars, the comparison between mature Namkokmai and Falan was carried out.

The structures of the lenticel of Falan are shown in Figs. 6 and 7. The lenticel of Falan had some different structural features from those of Namdokmai. That is, the lenticel protruded from fruit surface, cells constituting lenticel arranged rather regularly (Fig. 6), the cells at the boundary between lenticel and cortex had thick wall (Fig. 7), and the hole of lenticel was rather shallow.

Thick-walled cells existing at the boundary between lenticel and cortex showed green color with safranin and light green staining (Fig. 6) and greenish blue with toluidine blue 0 (Fig. 7). These cell walls were, therefore, considered not to be lignified but probably to be suberinized. To confirm that these cell walls became suberinized or not, however, it is needed to test the cells with, for example, Sudan blue (Purvis, 1964).

In Namdokmai the lenticel had no thick walled cells at the boundary between lenticel and cortex. It was, therefore, considered that the factors from outside reacted easily with cells in Namdokmai resulting in discoloration of lenticels.

3. The occurrence of discoloration in lenicels

Histochemical observation was carried out to consider the cause of discoloration of lenticels. In an unstained section cells constituting lenticel showed yellow or brown color (Fig. 8). The reason why the lenticels were observed as dark colored dots by naked eyes was because of the discolored cells.

Nitroso reaction (Reeve, 1951) was tested to clarify the components of colored substances (Fig. 9). This reaction indicated that these discolored cells contained polyphenols as one of the components. In detailed observation, however, the substances reacted to Nitroso reaction existed in vacuoles in the epidermal cells and cytoplasm in the cells of lenticel. The discoloration of cytoplasm in the cells of lenticel indicates that these cells were dead cells or under degradation.

In safranin and light green staining, the cells constituting lenticel showed dark red and the cell contents changed to debris-like structure different from the cells surrounding them which showed living condition (Fig. 10). This feature also indicates that the

cells of lenticel showed earlier necrosis than the surrounding cells.

From the histochemical observation, it was considered that the cells of lenticel showed earlier necrosis because some factors from outside, for example, air and water easily affected the lenticel. Cuticular layer which opened over the lenticel and the cells of lenticel which had many intercellular spaces and thin cell walls were considered to contribute the invasion of air or another factors into the lenticel. As the results cells reacted to air and the formation of polyphenols and colored substances took place.

It was also investigated whether the pathological factors related with the discoloration of lenticel or not. Fungi and hyphae were observed after cotton blue staining. No special distribution of them was observed in relation with lenticel. Although it is important to test the pathological factors not only from microscopy but also from biological method, no positive relationship between discoloration of lenticel and microorganisms was observed at least from light microscopical observation. Polyphenols existed in leniticel were considered to have the efficiency to prevent the invasion of microorganisms from outside because they are toxic substances (Friend, 1979).

In Falan lenticel did not show discoloration during maturation. This was considered because of the thick walled cells which made barrier to factors coming from outside. Lenticels of Falan had stronger structure than Namdokmai to the factors from outside.

In this report the anatomy of lenticels and their discoloration have been investigated. It was sometimes observed that some lenticels showed discoloration and others did not in one mango fruit though the physiological condition was almost the same. It is the future research point to clarify the reason of this phenomenon.

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