

THE SYMPTOMATOLOGY OF BLOSSOM END BROWNING (BEB) DISORDER ATTACKING ANNA APPLE FRUIT IN EGYPT

By

Hifny, H.A; Fahmy, M.A; Baghdady, G.A. and M.Z. Sultan
Horticultural Department, Faculty of Agriculture, Al-Azhar University-Cairo.

ABSTRACT: The present study was preformed in the two successive seasons 2006 and 2007 to reveal some facts about the blossom end browning phenomenon (BEB) in Anna apple fruits which causes high economic losses of total apple production in Egypt. Mature Anna apple fruits were harvested from six year old trees budded on M.M.106 root stock and grown in sandy soil in a private orchard. The fruits were after harvest immediately stored under room temperature ($26^{\circ}\text{C}\pm 2$) and inspected daily up to 10 days to follow up the apparent and histological changes during the incidence of BEB phenomenon.

The initiation of BEB which occurred three days after maturity, began with changing in peel color to become **strong yellow** in nearly circular watery spot, with 1-1.5 cm in diameter located aside of the fruit distal end (blossom end). In the fourth day; the attacked area circularly and slowly spread towards the proximal fruit end axis and also towards the fruit core axis to entailed one fourth of the fruit with " **dark yellow**" color of peel and flesh. The attacked area extended in the same way to cover one third of fruit mass on the 8th day with " **grayish brown**" color of the attacked tissue. Moreover, the attacked fruit mass which became more spongy and watery was secondary attacked with many fungal and bacterial micro-organisms on the 8th-10th day after maturity.

Regarding the histological aspects, the microscopic inspection of the attacked tissues elucidated that fruit tissues appeared normal and sound for two days after harvesting. On the third day after maturity some cells of the cortex tissue are shown with very thin walls, while some others appeared with deformed cell walls. On the fifth day after maturity, the deformed cells of cortex tissue increased and extended in two axis, those are towards the fruit core as well as towards the proximal fruit end. On the sixth day after maturity, the transverse section showed scattered group of cells in the cortex, which were completely collapsed. The damaged cell walls canceled the individuality of the cells, so that some elliptical grooves and cavities appeared in the cortex. Cell components began to vanish. The results suggested that the deformation of cortex cell

walls in the distal part of Anna apple fruit is the first step for BEB incidence.

INTRODUCTION

'Anna' apple (*Malus domestica* Borkh.) is the most dominating cultivar in all countries that have mild winter climate like Egypt because its chilling requirement for budbreak (less than 50 hours below 7.2 °C) (Childers, 1983, and Jackson, 2003). The total cultivated area of apple in Egypt reached 60.684 feddan in the seasons 2008, which produce about 550.000 tons /year .

Though, there are high economic losses of the production due to appearing the blossom end browning (BEB) phenomenon alone as post-harvest browning followed by decay of the apple fruit mass.

The available literature especially from mild winter countries did not reveal any information about this phenomenon. Hence, it seems that this phenomenon is a special case belongs to the egyptian environments as well as agricultural managements. Since no previous literature concerning the so called BEB phenomenon is available, we tried to make use of similar known phenomena that attack other kinds of fruits and are put among post-harvest disordered fruits.

There are a number of physiological disorders of apple fruits that can develop in the orchard or only become evident after harvest in the storage. Bitter pit is perhaps the most important of these disorders (Jackson, 2003). On the other-hand internal browning in apple fruit may appear after 3–4 months in cold storage and rapidly becomes more extensive when the fruit are removed to warmer locations (Moghaddam and DeEll 2008). Franck *et al.*,(2007), mentioned that internal browning disorder in pear (*Pyrus sp.*) fruits can be present in different forms such as radial browning, asymmetrical browning ,brown and/or dry spots cavities, brown core, etc. Ultra structural analysis of the brown area in the peel of many fruits showed hyperdermal tissue collapses and skin damage (Toivonen and Brummell, 2008). The visual symptoms of bitter pits development observed in apples started with cell protoplasm plasmolysis followed by plasma membrane breakdown and cell death (Sergio, *et al.*, 2010).

* According to the last census, issued by Ministry of Agric. - Egypt (2009).

Thus, the objective of this study is to follow up the phenomenon of blossom end browning (BEB) of Anna apples regarding its symptomatology including apparent and histological aspects during the initiation and development of BEB.

MATERIALS AND METHODS

The present study was carried out during the two successive seasons 2006 and 2007 in post-harvest and anatomy laboratories of Horticulture Dept Fac. Agri., Al-Azhar Uni. Cairo. Mature Anna apple fruits were harvested from six years old Anna apple trees (*Mullus domestica* L) budded on M.M.106 rootstock. The trees have grown in sandy soil in a private orchard located at El-Khatatba region, Monofeya Governorate. The trees were planted 3x4 meters apart and were irrigated through drip irrigation system. Maturity stage was adjusted according to a previous study of (ADS, 1982).

To follow up the progress and the spreading out of the BEB symptoms, beginning with time of initiation, 54 mature fruits were collected from nine trees (six fruits/tree). The trees were divided into 3 replicates x 3 trees per each. The fruits apparently showed healthy and uniform at time of picking. They were put in one layer in carton boxes under room temperature ($26^{\circ}\text{C}\pm 2$) and inspected daily for 10 days. Site of BEB initiation and its spreading through the fruit tissue were determined. Another 54 fruits (six fruits/tree) were collected from the same trees at maturity and were devoted to elucidate the changes occurred in fruit color either in peel or fruit flesh.

The colors of fruit peel and flesh: was determined using the available American color chart (Nickerson 1957).

Anatomical study was performed to determine the developmental stages of attacked tissues of Anna apple fruits with BEB disorder. Samples of both sound and attacked tissues were immediately killed and fixed in F.A.A. solution. Thereafter, the specimens were dehydrated using n-butanol method and embedded in paraffin wax. Horizontal sections with a thickness of 10 microns were prepared and stained with safranin and light green (Johanson, 1940). The permanent prepared slides were microscopically examined for identifying the initiation and development of BEB.

RESULTS AND DISCUSSIONS

Apparent inspection and determining the initiation of BEB:

Mature Anna apple fruits remained under observation in the lab at room temperature ($26^{\circ}\text{C}\pm 2$) for ten days. The fruits remained sound without any changing in peel or pulp color for two days after harvest (maturity). On the third day after maturity (as shown in table 1) the fruit showed some change in peel color and that was the initiation of the blossom end browning (BEB). Stages of the BEB-development are as follows:

Stage I: Browning initiation begins on the third day after maturity with a changing in peel and flesh color to become **strong yellow** in nearly circular watery spot, 1-1.5 cm in diameter, located aside of the fruit distal end. The coloration of the fruit flesh commenced in a spot completely similar to that of peel (fig. 1-a).

Stage II: On the fourth day after harvest, the coloration of attacked fruit parts of peel and flesh appeared "**dark yellow**". The attacked area, circularly and slowly spread towards the proximal fruit end axis and also towards the fruit core axis to entailed nearly one fourth of the fruit. The spreading of coloration was faster in the big sizes seedless fruits than in seeded small ones (fig. 1- b).

Stage III: On the fifth day after maturity, color of the attacked zone of fruit peel and flesh appeared "**dark orange yellow**" which entailed nearly one third of the distal fruit terminus. It is worthy to mention that the apple fruit appeared somewhat still acceptable for consuming, since the total browning of the distal fruit side is not yet be completed (fig. 1-c)

Stage IV: On the sixth day after maturity, color of peel and flesh developed to become "**moderate yellowish brown**". The coloration spread fast to include more than one third of the whole fruit at distal fruit terminus. The fruit becomes unacceptable for consuming (fig.1-d). The infection with fungal and bacterial organisms at distal dark brown tissues was appeared.

Stage V: On the seventh day after maturity, peel and flesh color developed to become "**moderate brown to dark brown**" at distal terminus of the fruit mass. The browning was concurred with the spongy

appearance of the colored mass at the distal one third of the fruit (fig. 1-e).

Stage VI: The last stage of the fruit deterioration begins on the 8th day and lasted to the ninth day after maturity. The color of the attacked fruit mass developed to become “grayish brown” on the 8th day. Coloration further developed to be dark brown on the 10th day after maturity. Moreover, the fruit mass which became more spongy and watery, was secondary attacked with many fungal and bacterial micro-organisms and the flesh mass at the distal half of the fruit appeared completely rot and deteriorated. Inspection and following up the mature Anna apple fruits in the two successive seasons 2006 & 2007 showed similar stages of the blossom end browning.

Table (1): Development of fruit browning, peel and pulp in BEB- disordered Anna apple fruits in 2006 and 2007 seasons.

Days after maturity of harvested fruits.	2006 season		2007 season	
	peel color	pulp color	peel color	pulp color
1	Appeared sound	Appeared sound	Appeared sound	Appeared sound
2	Appeared sound	Appeared sound	Appeared sound	Appeared sound
3	Strong yellow	Pale orange yellow	Strong yellow	Pale orange yellow
4	Dark yellow	Strong orange yellow	Dark yellow	Strong orange yellow
5	Dark orange yellow	Dark orange yellow	Dark orange yellow	Dark orange yellow
6	Moderate yellowish brown	Moderate yellowish brown	Moderate yellowish brown	Dark yellowish brown
7	Moderate brown	Moderate brown	Dark brown	Moderate brown
8	Grayish brown	Grayish brown	Grayish brown	Grayish brown

Fig. (1): Longitudinal cut in disordered Anna apple fruit shows the development of fruit browning in the storage for 10 days



Fig.(1-a) Stage (I):

Longitudinal cut in disordered Anna apple fruit shows the browning initiation. 1-1.5cm in diameter, nearly circular watery spot, aside of the fruit distal end, closed strong yellow in peel and flesh.

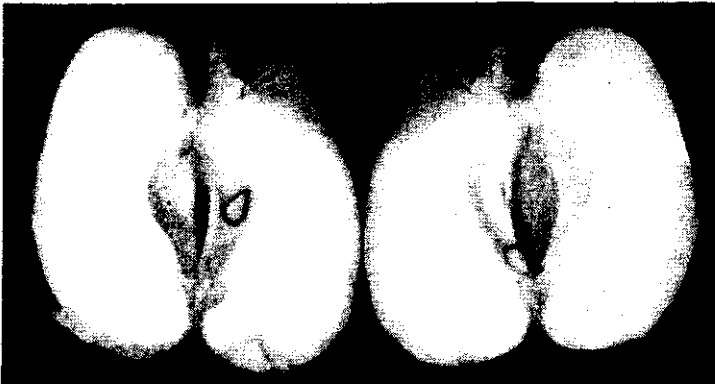


Fig. (1-b) Stage (II):

On the fourth day after harvest, coloration of attacked fruit part is “dark yellow”. The attacked area slowly spread to include one fourth of the fruit at the distal fruit terminus.



Fig.(1-c), Stage (III):

On the fifth day after harvest, color of attacked zones of peel and flesh appeared “dark orange yellow” and include nearly one third of the fruit at the distal terminus.



Fig.(1-d), Stage IV:

On the sixth day after maturity, color of peel and flesh developed to become “moderate yellowish brown”. More than one third of the fruit is attacked.



Fig.(1-e), Stage V:

On the seventh day after maturity, attacked zone in peel and flesh color developed to become “moderate brown to dark brown”. The browning is concurred with appearance of the spongy fruit mass. The dark brown tissues are secondary infected with fungal and bacterial organisms.



Fig.(1-f), Stage VI:

On the eighth day after maturity, attacked zone in peel and flesh color developed to become “Grayish brown”, fruit flesh became spongy. Coloration continuously developed to be dark brown on the 10th day. Secondary attack with many fungal and bacterial infection occurred at half of the fruit.

Similar symptoms were appeared in some other physiological disorders in several kinds of fruits such as:

A) Internal browning in pomes: which appeared in pear and became more severe during storage (Lammertyn *et al.*, 2003). It appeared in different forms such as radial browning, asymmetrical browning, brown and/or dry spots cavities, brown core, etc. Sound spots are often found in the extension of the five carpels through the brown zone. Cavities can be manifest in different ways: small spots in a star pattern in-between the five carpels or randomly localized dried lesions or randomly localized cavities, usually of a larger size (Franck *et al.*, 2007). Cavities eventually developed from brown tissue (Lammertyn *et al.*, 2003). The internal browning incidence in apple fruits rapidly appears and becomes more extensive when the fruits are exposed to warm climate during storage (Moghaddam and DeEll 2008). On the other-hand Moghaddam and DeEll (2008), elucidated that diffuse brown flesh in the apples cortex area was considered internal browning. Diffuse brown tissue in the core area next to the carpels is characteristic of core browning.

B) Blossom end rot (BER) in tomato and pepper fruits: which becomes visible first as one or more tiny lesions slightly depressed below the fruit surface at or near the blossom-end of the fruit and close to the base of the style. Later, the affected tissue appears watery and darker green. It reaches its full size in about one week from inception. Finally it shrinks or collapses, forming a depressed, leathery necrosis of the distal part of the placenta and the adjacent pericarp that gradually turns from brown to nearly black. External and internal BER may occur, affecting only tissues inside the outer wall of the fruit including some seeds (Petersen and Willumsen, 1992, Willumsen *et al.*, 1996 and Saure 2001). The initiation time of BER in tomato was recorded only at fruit age ranging from 12 to 15 days after anthesis. This period is characterized by maximal relative growth rate of fruit volume and the beginning of cell enlargement (Manishi *et al.*, 1996 and Saure, 2001).

Histological aspects of BEB disorder:

Elucidation: The manner in which the tissues and cells of the peel and flesh of mature Anna apple fruit are organized, might be the key of understanding how the cells structure rapidly collapsed specially at distal blossom end of the fruit, so that more than one third of the fruit completely appeared damaged in few days in blossom end browning

disordered apple fruit.

Microscopic slides of mature fruits as well as the development of browning of apple peel and flesh mass in Anna apple fruit in contrast to sound one has been prepared and followed up. All harvested mature fruits appeared sound on harvest day and had not any sign of browning attack. In the following a complete description of the tissue of sound fruit in comparison to that attacked with BEB disorder in addition to the developmental stages of fruit browning.

Mature sound fruit:

As can be seen in fig. (2), the transversal section in mature sound apple fruit shows the following tissues from outside towards the fruit core:

The epidermis: is a boundary tissue compressed of two layers of small epidermal cells. The outer epidermal layer is heavily coated with cutin (cuticle) and much wax, which deposited in the outer cell lamella and appeared irregularly striate.

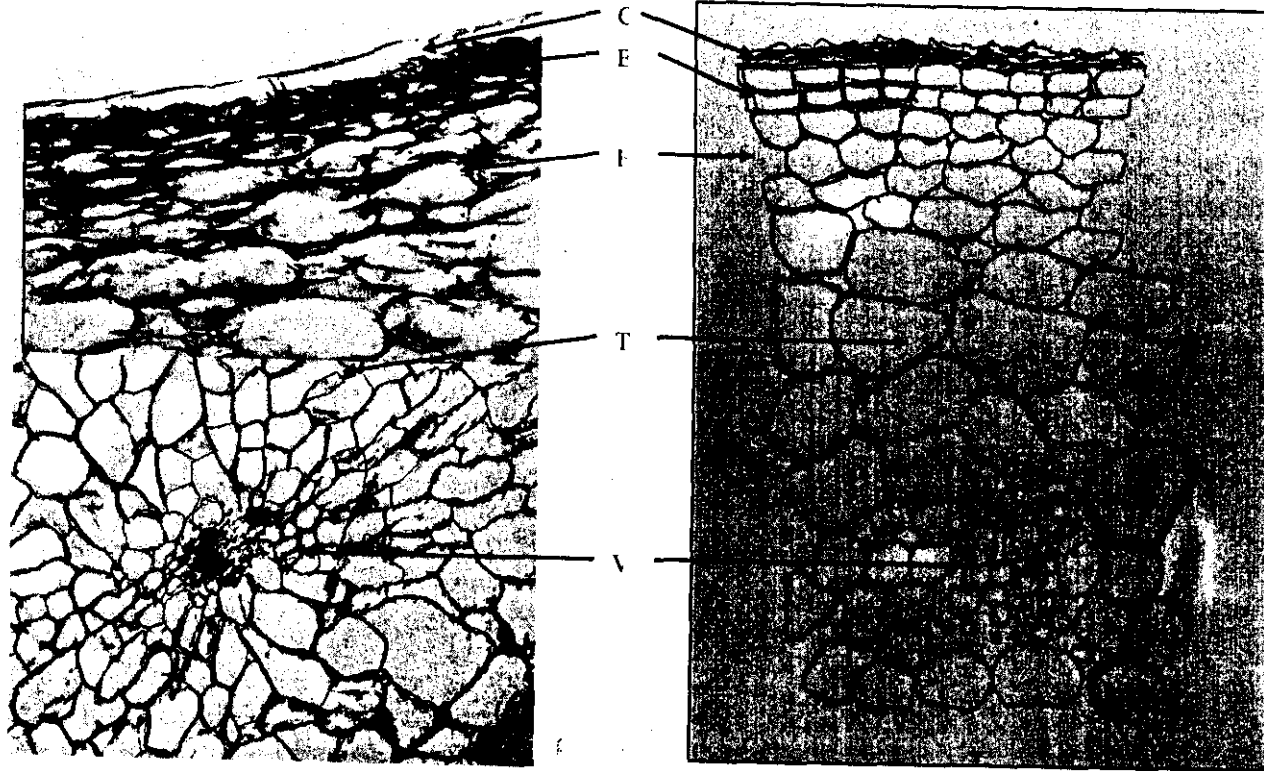
The hypodermis: appeared just below the epidermis tissue comprised of few layers (most are two layers), their cells sized small to medium and have tight packed thin walls. The **hypodermis** formed a transition zone between the epidermis and fruit flesh (the cortex).

The cortex (fruit flesh): is made up of large parenchyma cells having varied dimensions and directions. Parenchyma cells have thin walls and big vacuoles filled with cell sap. Cortex layers are many and they extend to fruit core.

The vascular tissue: vascular tissue extends from the core out and ram through the cortex in few to many strands ranging from small to large diameter strands. The vascular tissue is surrounded by a transition zone of cells which separate it from the cortex.

Anatomical feature of the BEB-disordered fruit of Anna apple:

Inspection of the microscopic transversal and longitudinal sections of Anna apple fruit remained for 10 days after harvest in the storage under room temperature elucidated that fruit tissues appeared normal and sound for two days after harvesting, which was performed at fruit maturity stage.



Transversal microscopic section in the cortex of mature sound fruit.

Schematic representation of the cortex tissues in mature sound fruit.

Fig. (2): Histological aspects of mature sound Anna apple fruit tissues.

C= cuticle

E= epidermis cells

H= hypodermis cells

T= transition zone

V= vascular tissue

Stage (I) the initiation of cell deformation: on the third day after maturity some cells of the cortex tissue (the flesh) of BEB-disordered Anna apple fruit are shown with very thin walls (too pale stained color), while some others appeared with deformed cell walls. Thus, cell deformation initiated in some outer cells of cortex at distal fruit end just below the hypodermis tissue. Nevertheless most cells of cortex tissue appeared normal. The epidermis tissue appeared normal; while some cells of hypodermis appeared more expanded than normal and have a pale staining color (fig. 3-a).

Stage (II): on the fifth day after maturity, the deformed cells of cortex tissue in disordered fruits increased and extended in two axis, namely towards the fruit core as well as towards the proximal fruit end. Some fruits showed in their microscopic inspection completely torn cell walls inside the cortex tissue (fig. 3-b).

Stage (III): on the sixth day after maturity, the transversal section showed scattered groups of cells in the cortex, which were completely collapsed. The damaged cell walls canceled the individuality of the cell, so that some elliptical grooves and cavities appeared in the cortex. Cell components began to vanish (fig.3-c).

Stage (IV): on the 8th day the cortex cells appeared completely damaged at distal fruit terminus. Cell walls appeared ruptured and scattered. Cell constituents completely vanished (fig. 3-d).

Similar histological aspects were reported by many workers about some physiological disorders of some fruit kinds. **Burdon et al., (1991)** reported that cell separation and cell wall degeneration occur in disordered mesocarp of mango fruit affected with internal breakdown, whereas cell cohesion is maintained in the healthy mesocarp.

Also **Raymond et al., (1998)**, found that in mango the first microscopic indicators of the disorder internal breakdown (jelly seed), is the disorganization of the cells and rupture of the cell walls followed by deterioration or dissolution of vascular connections between the stone and the mesocarp. Cross-section in sound fruits showed that the tissues of the fruit base had closely adhered cells, with xylem vessels intact and undisturbed.

Fig.(3): Transversal microscopic sections in the cortex tissues of BEB-disordered Anna apple fruit showing the developmental stages of fruit deterioration (x: 10 x 10).

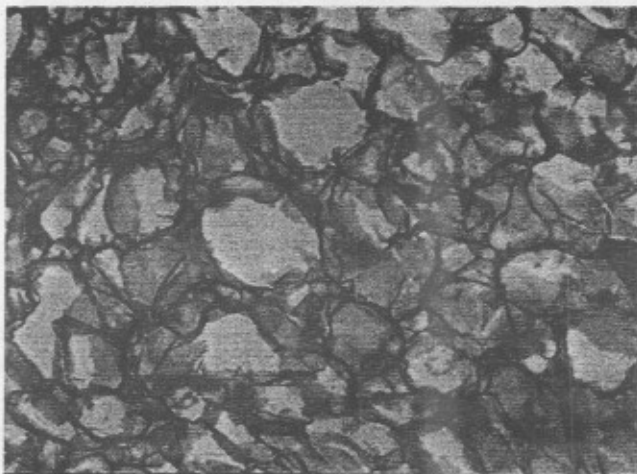


Fig.(3-a), Stage (I): Initiation of cell deformation. On the third day after maturity some cells of the cortex tissue are shown with very thin walls, while some others appeared with deformed cell walls.

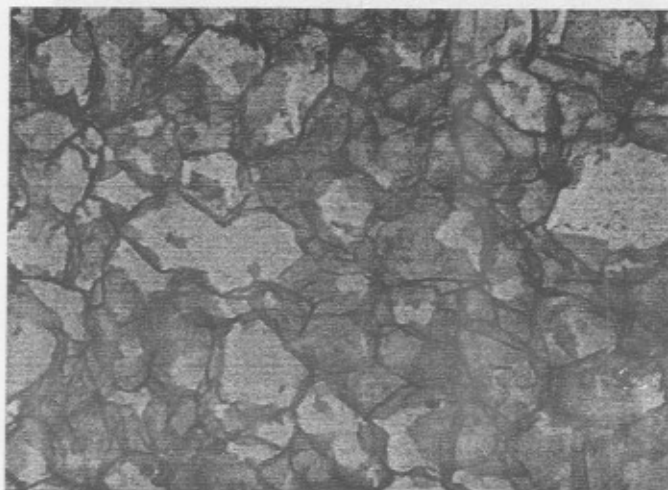


Fig.(3-b), Stage (II): On the fifth day after maturity, the deformed cells of cortex tissue increased and extended in two axis, namely towards the fruit core as well as towards the proximal fruit end.

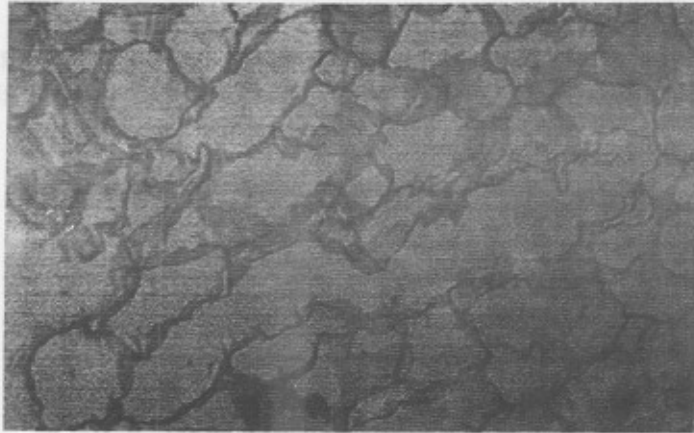


Fig.(3-c)Stage (III):

On the sixth day after maturity, the transversal section showed scattered group of cells in the cortex, which were completely collapsed.

Elliptical groves and cavities appeared in the cortex due to the damage of the cell walls

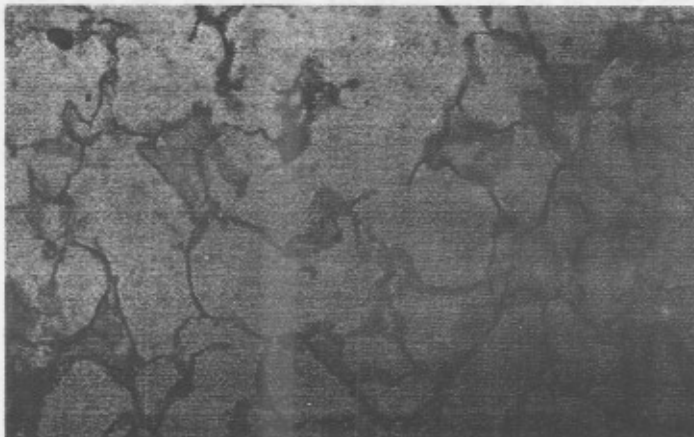


Fig.(3-d),Stage (IV):

On the eighth day after maturity, the transversal section showed scattered cell walls of the completely damaged cortex tissues at the distal disordered fruit terminus.

Jackson,(2003), reported that the first sign of bitter pit is the collapse of cell walls and by the time that pitting becomes visible to the naked eye. The pits cavities resulted from the collapse of several cells. Also **Sergio, et al., (2010)**, found that the visual symptoms of bitter pits development observed in apples started with plasmolysis followed by plasma membrane breakdown and cell death.

According to **Liu, et al., (2009)**, the ultra structural analysis of the brown area in apricot fruit showed that hypodermal tissue was collapsed and skin damaged.

We can resume that the microscopic inspection of the disordered apple fruit cross sections in the present study showed that collapsed cells initiated in the outer layer of cortex tissue, while the cells of the epidermis and hypodermis appeared in perfect cohesion and their walls are maintained in healthy structure.

On the other hand, the morphological inspection of the disordered apple fruit revealed that the BEB disorder began with changing of peel color, i.e. that the disorder initiated in the epidermis and hypodermis tissues. The two paradoxical images indicated that the incidence of collapsing outer cortex cells precede the browning appearance. Moreover, the rupture of the cell walls that occurred through cell collapse in the cortex might be the reason of tissue browning. Thus, the browning appeared in the fruit peel might be an advanced stage of the disorder preceded with collapse of epidermis and hypodermis tissues after deterioration of the cortex tissue. Therefore, it will be reasonable to throw the light on the mechanisms, by which the browning of the fruit tissues takes place.

Mechanisms of browning incidence:

To understand the mechanisms of browning incidence in BEB-disordered Anna apple fruit tissues it might throw the light on the synthesis and localization of phenolic compounds in the plant cell.

As shown in fig (4), the phenolic compounds are mainly localized in vacuole and the cell wall. Smaller quantities of phenolic compounds may be found in chromoplasts, cytoplasm and the mitochondria, but these are normally minute amounts (**Hrazdina and Wagner, 1985**).

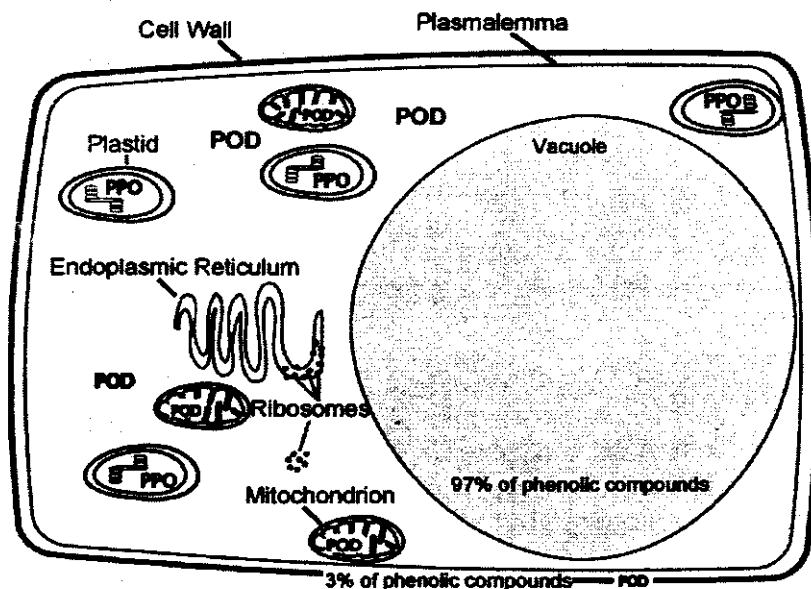


Fig. (4): The localization of phenolic compounds and phenolic oxidizing enzymes (polyphenol oxidase and peroxidase) in a typical plant cell (from **Toivonen and Brummell, 2008**).

POD: phenol peroxidase - PPO: polyphenol oxidase.

In intact cells, both phenolic compounds and browning enzymes are isolated by membranes which considered as barriers between them (**Toivonen, and Brummell, 2008**). Consequently, the initial step in the oxidative browning process must be the breakdown of membranes within cells of plant tissues (**Toivonen, and Stan, 2004**). The consequence of that step is the mixing of polyphenol substrates, i.e., catechin, polyphenols with polyphenol oxidase and/or phenol peroxidases (**Degl'Innocenti *et al.*, 2005**). We can assume that the collapse of the cortex cells of Anna apple fruit showed in the anatomical study of the present work already previously occurred to the tissue browning. The collapsed cells enabled the oxidizing enzymes PPO and POD to be indirect contact with the phenolic compounds located either in cell vacuole or those localized in plasmalemma (see fig.4). Thus the oxidation of phenolics will be completed to produce melanin, the brown substance. Therefore the membrane stability is potentially a major factor controlling the rate of browning (**Cantos *et al.*, 2002**). Thus the most widely used commercial anti-browning formulation available today uses calcium salts

and ascorbate (**Rupasinghe *et al.*, 2005**). Ascorbate is hypothesized to control PPO activity through its ability to reduce quinones to the native diphenols (**Nicolas *et al.*, 1994**). However, ascorbate has many other possible activities in tissues mainly inhibiting the browning, since it is a “universal” antioxidant (**Noctor and Foyer, 1998**) and can even quench lipid alkoxyl and peroxy radicals which involved in membrane deterioration (**Esp'ın *et al.*, 2000**).

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توصيف التلون البني الذي يصيب الطرف الزهري لثمار التفاح "انا" في مصر

حفني عبد العزيز حفني ومصطفى عبد الحميد فهمي و جلال عبد القادر بغدادي
ومصطفى نكي سلطان

قسم البساتين- كلية الزراعة- جامعة الازهر- القاهرة

الملخص العربي

أجرى هذا البحث خلال موسمين متتاليين ٢٠٠٦ و ٢٠٠٧ في معمل معاملات مابعد الحصاد والتشريح بقسم البساتين بزراعة الأزهر بمدينة نصر بالقاهرة. تم حصاد ثمار التفاح "انا" في مرحلة اكتمال النمو من مزرعة تفاح خاصة عمر ٦ سنوات مطعومة علي اصل M.M.106 ونامية في تربة رملية تروى بالتنقيط تقع في منطقة الخطاطبة. وقد خطط البحث لتوصيف ظاهرة التلون البني التي تحدث في قمة ثمار التفاح "انا" وتسبب فقد نسبة كبيرة من الانتاج. ووضحت النتائج ان التغيرات الظاهرية لحدوث وتطور التلون البني تحدث في اليوم الثالث بعد اكتمال النمو والحصاد وكانت أول التغيرات عبارة عن بقعة مائية ذات لون اصفر برتقالي باهت بقطر ١-١,٥ سم على أحد جانبي قمة الثمرة (الطرف الزهري). في اليوم الرابع من اكتمال النمو ازداد قطر البقعة تدريجيا في اتجاه دائري ولأسفل باتجاه قاعدة الثمرة وللداخل نحو مركز الثمرة واصبح لونها أصفر ثم زاد انتشار منطقة التلون البني في نفس الاتجاهات ليعم ثلث الثمرة تقريبا (الطرف الزهري) الذي اصبح لون انسجته بني رمادي وفي اليوم العاشر اصبح لون الانسجة بني مسود وظهرت عليها الاعفان.

بالنسبة للتغيرات التشريحية اظهرت النتائج ان اول الملامح التشريحية للانسجة المصابة تظهر في اليوم الثالث بعد اكتمال النمو حيث لوحظ ان جدر خلايا الانسجة المصابة اصبحت رقيقة والبعض تشوه ، اما خلايا القشرة فبدت سليمة وظهرت خلايا الهيودرمس ذات حجم اكبر من الطبيعي .

في اليوم الخامس من اكتمال النمو ازداد عدد الخلايا المشوهة ذات الجدر الممزقة في الانسجة المتلونة باللون البني. في اليوم السادس من اكتمال النمو، ظهرت مجموعات من الخلايا المتناثرة منهاره الجدر (متبلزمة) ، كما ظهرت تجاوب في الانسجة نتيجة تلاشي جدر الخلايا وافتتاح الخلايا علي بعضها حيث لم يصبح لكل خلية استقلالها بجدار يوضح ملامحها وتلاشت مكونات معظم الخلايا الداخلية.