Chilling injury in mangoes

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To my parents

My wife and my little wild flowers

(Lojina & Norseen)

Content

Chapter 1

General Introduction

Preamble

Mangoes are a popular, nutritional tropical fruit, which are now one of the most important fruits crops in tropical and subtropical areas of the world. They originated in India, were they have been cultivated for more than 4000 years. Beginning in the16th Century, mangoes were gradually distributed from India to other tropical countries in Asia such as the Philippines, Indonesia, China and Thailand. They were also spread to the Americas in the 18th Century, and from western Mexico they were carried to Hawaii in the early 19th century. The first recorded introduction to Florida was at Cape Sable in 1833. Mango was introduced to Egypt in the 18th Century from Ceylon by the Egyptian leader Ahmed Orabi upon his release from internment. Many cultivars grown in Egypt today, such as 'Zibdia' and 'Hindi Be-Sennara' date form this time (Ibrahim and Khalif, 1999) Figure 1.

Increasing commercial acreage, and improved handling shipping procedures are expected to increase the global market penetration of the fruits. Currently, the major producers include India, Pakistan, Indonesia, Mexico, Brazil, and the Philippines. Other important producers are Australia, South Africa, Egypt and U.S (Crane and Campbell, 1994).

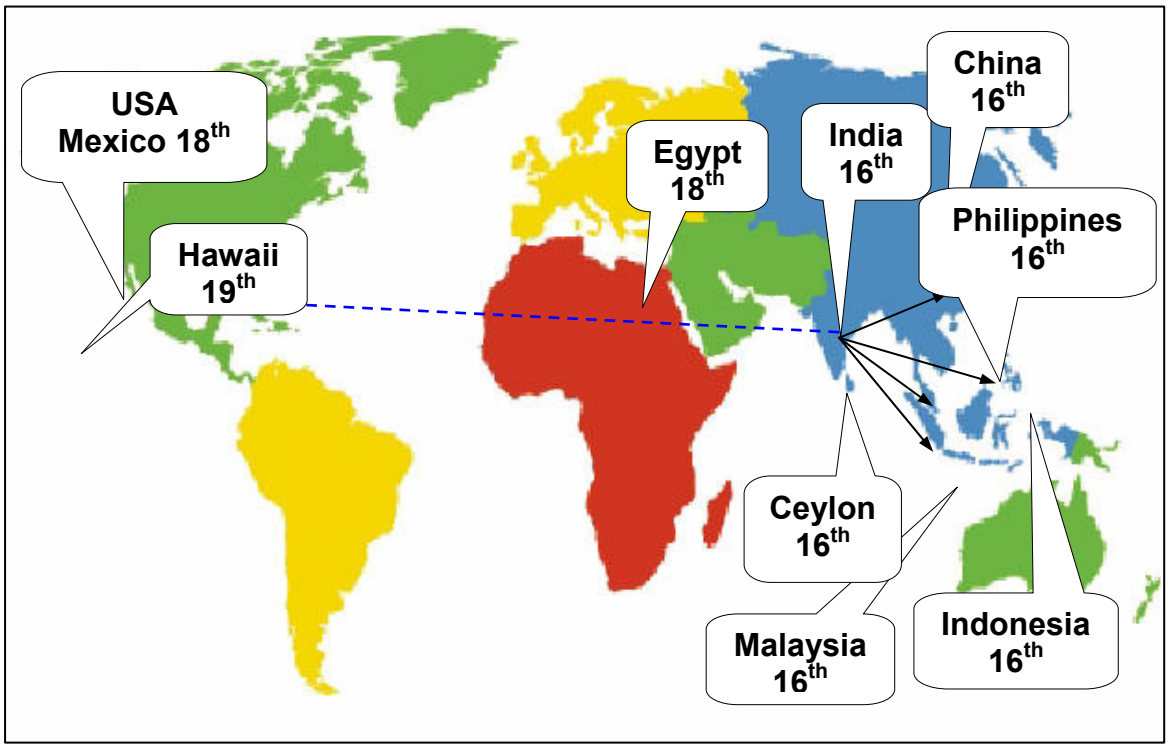

Figure1. The spread of mango from the original region of cultivation (India) round the world

Botanical and taxonomical of mango

The mango tree is a medium to large (9.1 to 30.5 m) evergreen tree with a symmetrical, round canopy that ranges from low and dense to upright and open (Figure 2). Leaves are alternately arranged, lanceolate, 15 to 40 cm in length, and leathery in texture. Their colour is pinkish amber or pale green-coloured when young, becoming dark green at maturity (Figure 3). The inflorescence is a many branched panicle borne at the end of the shoot, it is 6 to 40 cm in length and possesses from 550 to more than 4000 flowers. These are small, pinkish-white, and mostly staminate (Figure.4). The fruits are classified as drupes and they vary in shape (nearly round, oval, ovoid-oblong), size (from few 100s of grams to more than 2.5 Kg), and colour (greenish, greenish-yellow, yellow, red, orange, or purple) depending upon the variety. The skin is smooth and leathery and surrounds a fleshy, pale-yellow to deep-orange edible pulp. The fruits possess a signal large, flattened, kidney-shaped seed that is enclosed in a woody husk. Although the fruits will ripen on the tree, commercially they are usually picked when firm and green for shipment to the market. The crop is considered mature when the shoulder of the fruit broadens (or fills out) and some fruits on the tree have begun to change colour from green to yellow (e.g. Pride, Irwin and Tommy Atkins,) (Litz, 1997). Prior to this external colour break, the fruit is considered mature when the flesh near the seed changes colour from white to yellow (Figure 5). Mango is harvested in three maturity stages: immature (no shoulder development and of poor quality), half-mature (the shoulder has developed to reach the same level as the stem insertion point on the fruit) and full mature fruits (the shoulder has filled out and extends to beyond the stem insertion point; this is the optimum quality) (Majeed and Jeffery, 2002; Mitra and Baldwin, 1997). Taxonomically, mangoes belong to the genus *Mangifera.* It is one of the 73 genera*,* which include around 850 species, of the family Anacardiaceae in the order Sapindales.

Figure 2. Tree

Figure 3. Different leaves maturity

Figure 4. Flower cluster with round 5000 flowers

Figure 5. Different size and colure of mango fruits which are related to different species.

The 69 *Mangifera* species that have so far been recognized are restricted to tropical Asia.

Kingdom: Plantae (Plants)
Subkingdom: Tracheobionta (Vascular plants)
Superdivision: Spermatophyta (Seed plants)
Division: Magnoliophyta (Flowering plants)
Class: Magnoliopsida (Dicotyledons)
Subclass: Rosidae
Order: Sapindales
Family: Anacardiaceae (Sumac family)
Genus: *Mangifera* L.
Species**: *Mangifera indica* L.**

The Annual Global and Egyptian production of mangoes

Global production

Globally, mango production is increasing. In 2003, over 25 million tonnes of mangoes were produced from about 90 countries. Asia was the main producer with 76.9% of the total world production, followed by the Americas with 13.8%, Africa with 9% and less than 1% for Oceania (Table 1).

Table1. Distribution of world production during four years by continents (million tonnes)(Source: www.fao.org)

Continent	2000	2001	2002	2003
Asia	18.65	18.66	19.95	19.06
America	2.51	2.53	2.49	2.50
Africa	2.50	2.64	2.62	2.63
Oceania	0.05	0.04	0.03	0.03
Total	**23.70**	**23.87**	**25.09**	**24.22**

Nine countries together produced 83% of the total mango production in 2003 (Table 2), of which India was the most important (10 million tonnes) followed by: China, Thailand, Mexico, Pakistan, Indonesia, Philippines, Nigeria and Brazil.

Table 2. The total production of main mango producing countries during the four year period from 2000 to 2003 (Source www.fao.org)

Countries	Production (10^3t)				
	2000	**2001**	**2002**	**2003**	**2004**
India	10500	10240	10640	10500	10800
China	5211	3273	3513	3413	3622
Thailand	1633	1700	1750	1750	1750
Mexico	1559	155	1523	1503	1503
Pakistan	990	1037	1036	1036	1072
Indonesia	876	923	1403	731	800
Philippines	848	881	956	890	890
Nigeria	730	730	730	730	730
Brazil	538	782	842	845	845

Egyptian production:

The total mango production of Egypt was 197 million tonnes in 1997. The majority of mango production (97.5% =192 million tonnes) is produced from by the Nile valley provinces where most of the production area is concentrated in the provinces of Sharkia, Ismaillia, Gisa, Fayuom, Qena and Nobaria, which produced respectively 75, 37, 36, 17, 6 and 4.5 million tonnes each (Mamdouh, 1997) (Figure 6). The most important mango cultivars in the Egyptian market are, Hindi Be-Sennara, Pairi, Tymour, Ewas, Zibida and langara. Mangoes are the sixth most important fruit in the Egyptian market after citrus, grapes, olives, apples, and banana (Mamdouh, 1997).

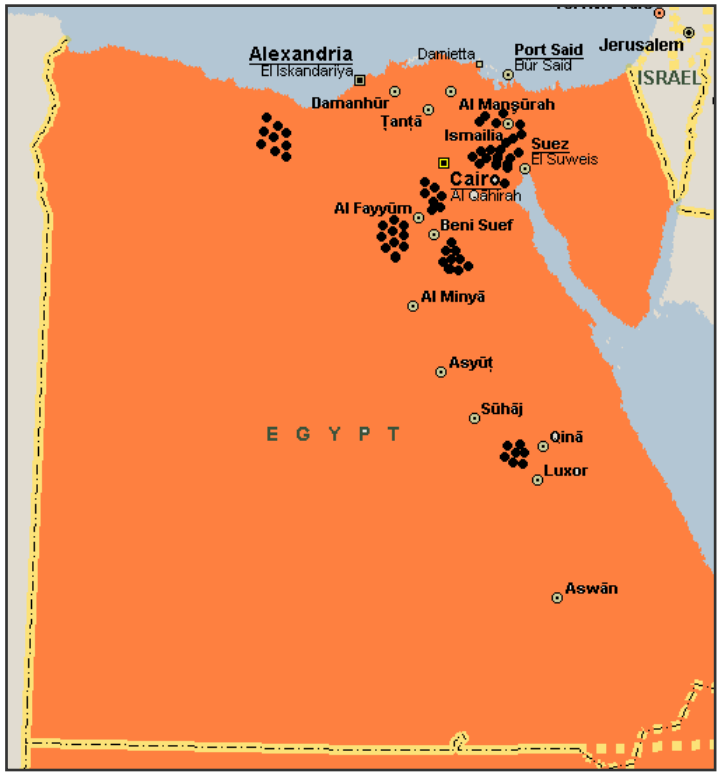

Figure 6. Distribution mango in 6 major provinces in Egypt.

Recently, according to the FAO organization (www.fao.com) (Figures 7 and 8). the total harvesting area and production increased last four year (2000-2004)

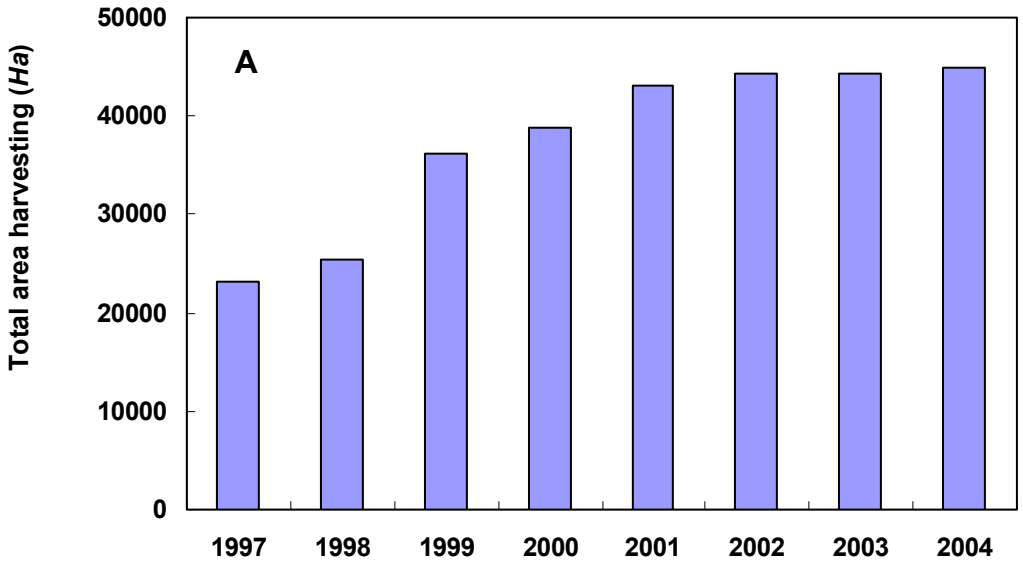

Figure 7. Egyptian total area harvesting during last four years (1997to 2004)

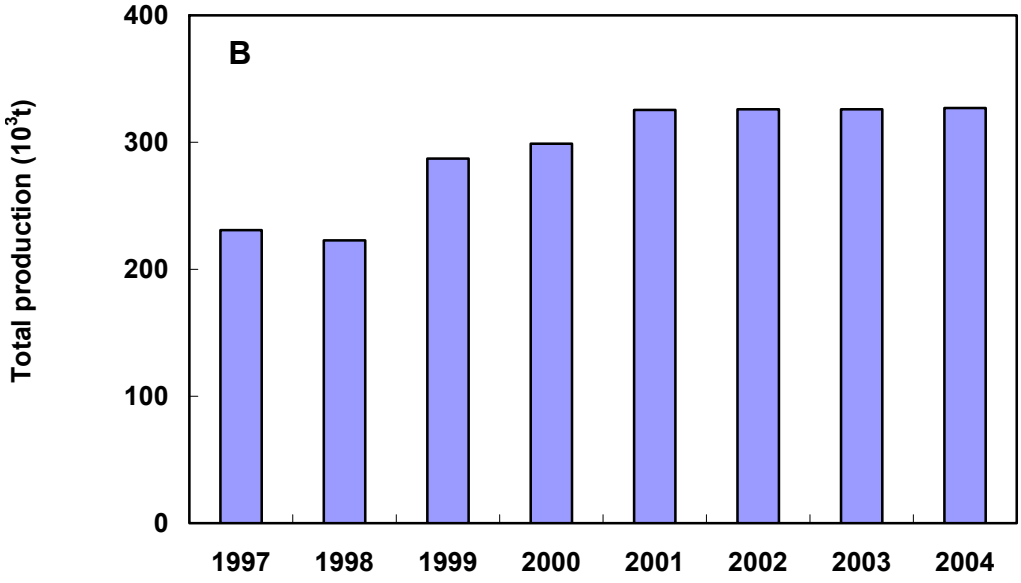

Figure 8. Total production 10^3 t during four years (2000 to 2004)

Mango market

The European Union mango market has developed greatly between 2000 and 2003, increasing by almost 10 million tonnes, and the growth in Mango consumption is expected to continue to grow. The fruit is imported from many countries, with Brazil being the main supplier with 33% of EU market in the year 2000 (Saúco, 2002). The increase in imports coupled with improved transport and storage techniques has lead to the year round availability of mango in Europe but the down-side for the producer is a lower price (Table 3). Egypt exports mangoes to Europe, with England and France being the main importers (Information and Decision Support Center 2003) However the Egyptian market also extends to Arab countries such as Dubai, Saudi Arabia, Bahrain, and other Gulf countries. In order to meet the growing demands from the Arab Gulf states and the EU the Egyptian Agricultural ministry plans to increase both mango production and the total plantation area during the coming 10 years. Moreover, many new mango cultivars, such as Kent and Tommy Atkins, are being introduced from other countries (e.g. India and South America) into Egypt.

Table 3. Mango imports into European countries (source www.fao.org)

Countries	2000		2001		2002		2003	
	Mt	Value t^{-1} (10^3 $)	*Mt*	Value t^{-1} (10^3 $)	*Mt*	Value t^{-1} ($10^3$$)	*Mt*	Value t^{-1} ($10^3$$)
U. kingdom	22017	25.98	26957	30.37	24235	26.097	31933	39.555
France	26262	29.96	25693	35.71	26833	30.695	32299	58.404
Netherlands	61856	67.18	69566	68.66	71479	70.907	91133	112.519
Germany	23321	24.93	24825	27.78	27954	29.659	31937	37.09
Belgium	16118	18.07	10292	10.77	10319	11.036	10824	13.597
Portugal	9548	11.3	15189	14.97	15938	14.499	19639	20.696
Switzerland	2794	4.99	3798	6.38	3973	7.372	4808	10.118
Austria	2513	2.46	2438	2.83	2682	3.214	4113	4.224
Spain	9188	10.87	7231	7.64	10410	12.587	11938	15.646
Total	**173617**	**195.74**	**185989**	**205.111**	**193823**	**206.066**	**238.624**	**311.849**

Mango problems:

The key features relevant to the profitability of mango production are the identification of mango market demand and its seasonality prices, seasonal fluctuations in availability, supply competitors, importer requirements, the option of added value processing, and sales promotions: the balance between these components will determine product price (Litz, 1997). To optimize mango production (harvesting, transferring, shipping, packaging and handling), we need to focus on production in relation to problems encountered throughout the chain as a whole. The practical problems relating to mango are conveniently classified into those which are largely pre-harvest in nature and those that are largely post-harvest.

Pre-harvest factors:

1. Production seasons are short (2 weeks to 2 months).
2. Varietal appearance and flavour are diverse.
3. Maturation time is variable.
4. Fruit abortion and premature fruit abscission results in yield decreases.
5. Irregular flowering leads to irregular fruiting.
6. There is year to year fluctuation in production

Post-harvest factors:

1. Overall the shelf-life is short.
2. Post-harvest, mangoes ripen rapidly.

3. Mango is sensitive to storage temperatures below 12^{o}C.
4. Anthracnose (*Colletotrichum gloeosporoides*) is the most important post-harvest disease, causing huge losses and wastage of mangoes.

The factors that determined the post-harvest behaviour of mangoes are illustrated in figure 8. They include from farm management during the production phase, the time of harvest and the availability of the appropriate technology to store and extend the shelf life of the fruits. The post-harvest performance of the fruits will be substantially determined by the action of processes that give rise to ripening and senescence during the post-harvest phase. The activity of these processes is the result of the interaction of biophysical, biochemical and developmental processes occurring in the fruits with their physical environment. Thus the appropriate physical environment can delay ripening and thus increase shelf-life, whereas the incorrect physical environment will accelerate ripening or cause other forms of product quality loss, and thus decrease shelf-life. Therefore, understanding the interaction between the physical environment and product biology is essential if product storage is optimized. However, not much is known about the biochemical and biophysical processes of the fruit upon which environmental factors act.

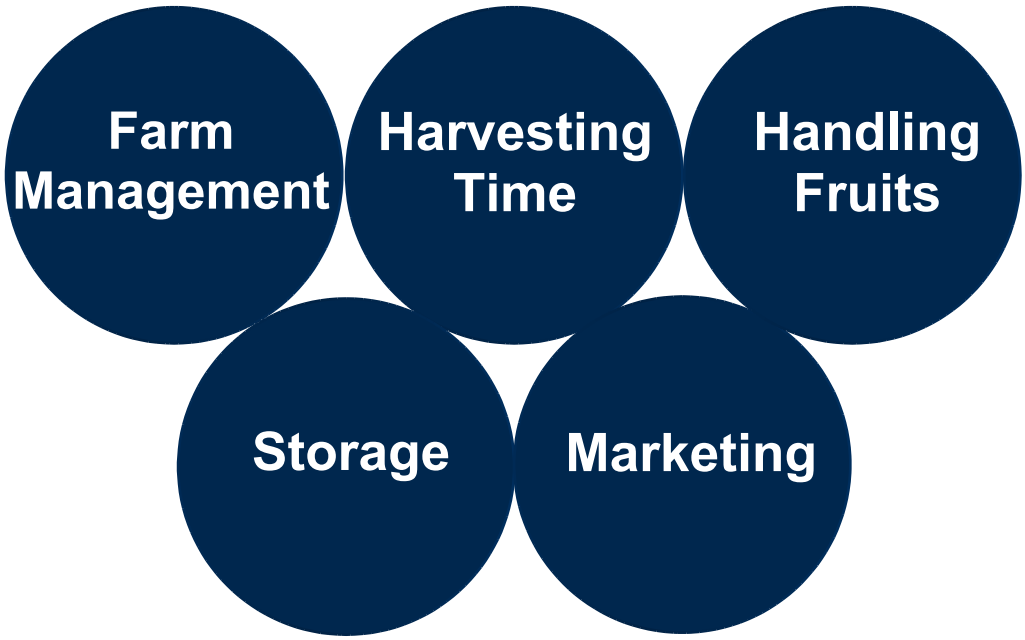

Figure 8. Pre and post harvest factors are affected on fruits quality

Aim of this thesis

Mangoes, the fruits of the tropical tree *Mangifera indica,* have a global production which places them amongst the ten most important fruits in the world. However in contrast to other major fruits, such as apples, pears or various species of citrus, the development of appropriate post-harvest technologies for mangoes has been only poorly studied and now lags a long way behind that of the other fruits. This results in problems in the storage and transport of mangoes, and thus less profit and restricted export opportunities for mango producers. Mangoes are a tropical product and many of the producer countries are classed as underdeveloped, so increasing the market for mangoes by means of improved post-harvest management could offer much needed economic opportunities for these countries.

This thesis will deal with various aspects of the post-harvest physiology of mango. During the post-harvest phase fruits and other products are subjected to a range of treatments and conditions as a result of transportation, handling, sorting, cleaning, storage, packaging, retailing etc. Depending on the perishability of the product, it will inevitably deteriorate more or less rapidly during the post-harvest phase as a result of the processes of ripening and senescence. Depending on the post-harvest conditions and processes this deterioration can be substantially delayed or accelerated. Thus understanding how postharvest conditions affect the natural processes of ripening and senescence. A major aim of post-harvest research is therefore to improve upon our understanding of how this fruit responds to storage under various conditions so as devise better means of storing mangoes and measuring the changes in product quality during storage.

The post-harvest phase succeeds production, and though it is often considered independent of it, this approach is dated and oversimplistic. Current models of post-harvest quality often attempt to relate post-harvest phenomena with pre-harvest conditions. This approach recognizes that as the post-harvest phase succeeds the pre-harvest phase, then practices in the former will have a major influence on the product performance post-harvest. For any cultivar, post-harvest behaviour therefore ultimately depends on the pre-harvest growth conditions of the product, and subsequently how that product responds to the post-harvest treatment it experiences. To explore these relationships in perennial woody plants (compared to shorter lived herbaceous crops) is not easy owing to the size of the plants; this produces difficulties in environmental control, coupled with cost of any treatments applied and the plants themselves. So the dependency of post-harvest mango fruit responses upon pre-harvest conditions in addition to the post-harvest environment is still relatively poorly understood compared to products originating in cool-

temperate or warm-temperate regions. This study will be restricted to investigating how maturity stage at harvest affects the post-harvest behaviour of mangoes, especially in relation to their anti-oxidant levels and cell injury developing under low temperature storage conditions.

Scope of the thesis

Chilling stress during storage leads to typical visible symptoms in various tissues and disruption of normal physiological function, thereby causing metabolic imbalance which are often used to quantify and characterize chilling injury development (Walker et al., 1990). In other words, chilling injured fruits suffer from physical and physiological changes induced by low temperatures and each commodity display characteristic symptoms. These symptoms are a function of plant species, degree of maturity, storage time and storage temperature and other environmental conditions (Wang, 1990).

Mango fruits are sensitive to low storage temperatures (below 12^{o}C), which plays a key role in the physiological reactions in fruits that lead to lower metabolic reaction rates at the chilling temperatures. The most common visible symptoms of chilling injury in mango fruits are dark skin, scald-like discoloration, and pitting or sunken lesions on peel. Even abnormal ripening and decay can be enhanced when mango fruits are exposed to temperatures below 12^{o}C (Chaplin et al., 1991). For this reason, storage of mango fruits at low temperatures limits safe transporting, shipping and marketing of mango fruits.

This thesis aims to study the phenomenon of chilling injury in mango fruits and the various factors affecting its development. Two Egyptian mango cultivars, Zibdia and Hindi Be-Sennara, were used as models for this research. The former is chilling sensitive while the other is relatively resistant to chilling conditions. Chilling determining conditions such as the fruit maturity, storage time and storage temperature were varied in order to get an insight into the mechanism of chilling phenomenon in chapter 2. The development of chilling injury symptoms in both mango cultivars and the variation of chlorophyll fluorescence will be presented in Chapter 3. Development of chilling injury in fruits is caused by the activities of antioxidants in fruits. Antioxidants can be classified into water and lipids-soluble antioxidants such as ascorbic acid, α-tocopherol and β-carotene and antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase will be studied in chapter 4 and chapter 5, respectively. The physiological changes occurring during storage of mangoes such as ethylene production, respiration rate, fruit firmness, and ion leakage of fruits will be studied in chapter 6. Thereafter, we aim to present the physiological background of chilling

injury phenomena in next parts to aware the input and output metabolic reaction during long storage.

Chapter 2

Chilling injury and the balance between active oxygen species and the scavenging capacity: a review

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Abstract

Chilling injury occurs when certain plants, or organs thereafter, are exposed to temperatures below 15^{o}C, but above the freezing point of the tissue. After a certain period of storage at these temperatures, and especially after a subsequent rise in temperatures, certain injuries in the plant tissue can be seen, such as water soaking, pitting, or browning. The possible causes of this type of injury will be reviewed and the consequences for cell components summarized. A distinction is made between the primary processes of chilling injury and subsequent secondary injuries. The membrane phase change and oxidative stress models of primary chilling injury are introduced. How cell components are affected by chilling injury is then discussed, as is the relationship between pre-harvest conditions, post-harvest handling, and injury. Lastly, the roles of different anti-oxidants and anti-oxidant enzymes are discussed and some techniques used for the measurement of chilling injury are introduced.

Chilling injury

Cold storage slows respiration and other metabolic processes that lead to ripening and senescence of fruits and vegetables during storage. So in order to extend their shelf life, most fruits and vegetables are cooled as rapidly as possible after harvest and stored at low temperatures until they are consumed. However, the shelf life of many fruits and vegetables, especially those of tropical and subtropical origins (such as mango fruits), as well as some of temperate origin, are actually shortened when they are stored at low temperatures. For examples banana and avocado are sensitive to low storage temperatures while green beans, lima beans, melons or peppers spoil more quickly at low than high temperature (Paull, 1990). Injury resulting due to exposure to low temperatures above freezing is termed chilling injury (CI) (Purvis, 2004). CI is an economically important post-harvest problem that reduces the overall quality and marketability of many harvested fruits and vegetables indigenous to the tropics and subtropics (Mohammed and Barthwaite, 2000) Chilling injury may occur in transit or distribution, in retail or storage (Mitra and Baldwin, 1997). At low storage temperatures, various physiological and biochemical changes occur in the product's tissues in response to chilling temperature. These are categorized as CI. The symptoms of CI vary depending on the plant species and tissue type and the severity and duration of the exposure to low temperature. The symptoms usually developed more strongly once the product is returned to non-chilling temperatures. The extent of chilling injury damage is hard to quantify and the extent or degree of damage is commonly only qualitatively or semi-quantitatively estimated using visual inspection of the product, (e.g. the chilling injury index (Chaplin et al., 1991).

Chilling temperatures induce various structural changes in cell structure and they disrupt a number of metabolic processes. Consequently several mechanisms of CI have been proposed. To try and create a rational framework within which chilling injury could be understood, (Raison and Lyons, 1986) defined CI as the physical and/or physiological changes that are induced by exposure to low, chilling temperatures. These physiological changes may be considered primary or secondary injury. A primary injury is the initial rapid response to low temperature that causes a dysfunction in plant cell or metabolic process. They considered that these primary injuries are readily reversible if the temperature is raised to non-chilling conditions. Secondary injuries arise as a consequence of the primary injuries, but unlike primary injuries, secondary injuries may be irreversible and thus they can lead to permanent damage or death whatever the subsequent handling of the product. A difficulty that has challenged analyses of the origins of chilling injury

is the diversity of processes or mechanisms that have been implicated in its cause, and the difficulty of reliably distinguishing primary from secondary injuries.

Chilling injury is not the same as freezing injury, which is the result of damage due to cellular dehydration following extracellular freezing of water and damage from the ice-crystals that then develop. Freezing damage can only develop when tissues are held at temperatures below their freezing point. The minimum safe temperature for chilling sensitive commodities will be substantially above their freezing point. The critical temperature at which chilling injury symptoms develop depends upon genetic factors, such as the species and cultivar, and physiological factors, such as the tissue type, its recent history and its physiological condition. For a mango fruit the freezing point will be about -1.4^{o}C (Kader, 2002), but storage temperatures below 12^{o}C are considered unsafe for these fruits (Mitra and Baldwin, 1997). Mangoes, therefore often have a short storage life, as low temperatures cannot be used to slow deterioration and pathogen growth.

1. Chilling injury symptoms in Mango

Mango fruits are considered to be climacteric* and they ripen rapidly after harvest. The storage, handling and transport potential of fruits is limited by susceptibility to diseases, sensitivity to storage temperatures below 12^{o}C, and perishability due to ripening and softening (Acosta et al., 2000). The combination of storage temperature and duration of storage are considered to be the important factors that lead to chilling induced physiological and metabolic dysfunctions in plant cells. These dysfunctions lead to various visible disorders that are commonly used to assess the degree of chilling injury experienced by the fruits (Walker et al., 1990). In mangoes, the most common visual symptoms are dark, scald-like discolorations in the peel, beginning around lenticels and spreading outwards to produce a more or less circular lesion, pitting on the fruit peel, the development of off-flavours, discolouration of the pulp, and overall poor fruit quality (Nair et al., 2003) (Figure 1).

* Climacteric: a sudden increase respiratory activity that is correlated with a sudden increase in the ripening process and a maximum in ethylene production associated with an autocatalytic ethylene production

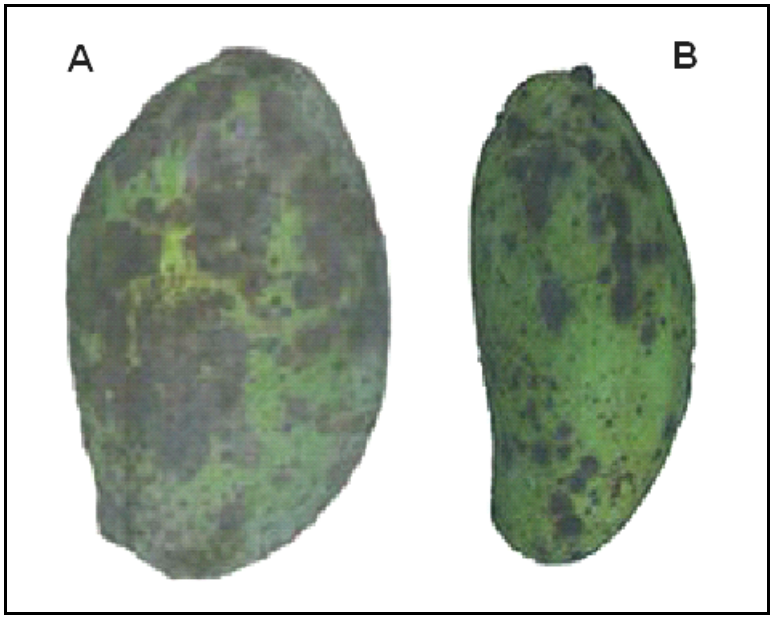


Figure 1. Severe chilling injury symptoms of Zibdia (a) and Hindi (b) during storage at 4^{o}C for 25 days

2. Chilling injury and its mechanism: the bilayer membrane

The instability of lipid bilayer membranes at low temperatures has long been thought to be a possible cause of chilling injury. Lipid bilayers are deceptive structures. Though they seem structurally simple, this belies an underlying complexity of composition and dynamics. The physiological and structural properties of the bilayer depend upon its organization, and this depends upon its composition. Bilayers are comprised of mixtures of numerous types of lipids and proteins, and not all of the lipids found in cell membranes will naturally form bilayers in the pure form at physiological temperatures. As temperatures decrease lipid bilayers will change from their normal flexible liquid-crystalline and functional state to a more solid gel-like structure. This solidifying of the membranes results in a decrease in membrane flexibility so cracking can occur and channels can form at the liquid-crystal/gel interface. These cracks and channels lead to membrane leakiness, a loss of membrane integrity, and the loss of solute or ion gradients across the membrane. Further, the transition from the liquid-crystalline to gel phase produces changes in the stability of the remaining mixture of lipids that comprise the liquid-crystalline membrane. The gel-phase regions of membrane that form at low temperatures are crystals that may contain only one lipid species, so the remaining liquid-crystalline phase will now be enriched in some lipid species and proteins and deficient in the species crystallised in the gel phase regions. As a result of the change in lipid composition the remaining liquid-crystal phase will have different structural and physical properties than the original lipid mixture. The

solidification and phase separation of the membranes also disrupts normal functioning of membrane bound proteins owing to the change in the lipid environment of the protein. The reaction rate of these membrane-bound enzyme systems is inhibited leading to an imbalance in the plant's metabolism, and the accumulation of metabolic intermediates which could be toxic to the plant. Leshem, (1992) has summarized the physiological and biochemical changes due to low temperatures in plant tissue (Figure 2).

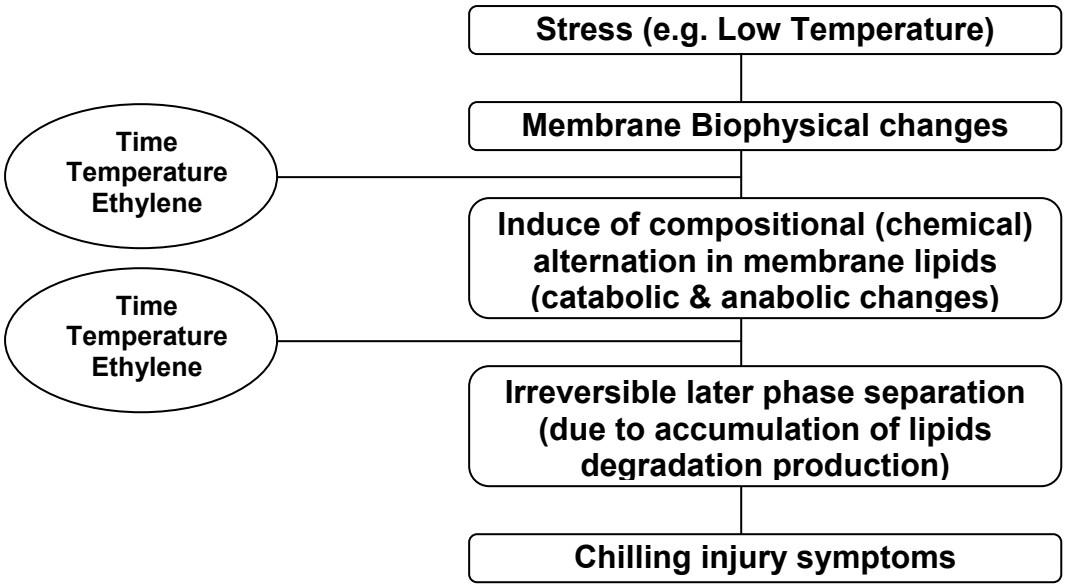


Figure 2. A model for the sequence of metabolic events that leads from the stress-induced alteration in the properties of membranes to the observation of macroscopic tissue damage

3. Chilling injury and its mechanism: Oxidative stress

In addition to the role for membrane dysfunction at low temperatures, it is also becoming widely accepted that the symptoms developing during or after storage at low temperatures (and also many other environmental stresses) are actually a consequence of oxidative stress in the tissues (Scandalios, 1993). The degree of oxidative injury resulting from abiotic and biotic stress imposed on plant tissues will depend on the one hand upon the oxidative challenge that arises in the stressed tissue, and on the other upon the cellular capacity to eliminate or deal with this challenge.

Oxidative stress is due principally to active oxygen species (AOS) derived from molecular dioxygen. There are various forms of AOS which are formed in various ways, and the means of formation limits which kind of AOS can be found in particular tissues under particular circumstances. For example, the formation of singlet state dioxygen is nearly always dependent on photophysical processes and

thus will only occur in light and in the presence of a photophysical sensitiser, such as chlorophyll. So in contrast to leaves in sunlight, singlet oxygen is unlikely to be a problem for fruits in dark storage: there is no light and the bulk of the tissue usually lacks any senstiser. On the other hand, the radical anion superoxide ($O_2^{\bullet -}$) is formed by the reduction of molecular oxygen (En=-140 mV) (Sun and Trumpower, 2003), and this can occur widely throughout the cell as a parasitic process associated with formation of reduced cofactors in metabolism.

Most (85% to 95%) (Liu and Huang, 1996; Purvis, 2004) of the oxygen consumed by non-photosynthesizing or non-photosynthetic plant tissues is reduced to water by the terminal oxidase(s) of the respiratory electron transport chain in the mitochondria. The remaining 5% to 15% is partially or fully reduced or incorporated into organic molecules by various other oxidases and oxygenases. Some electrons from single-electron-reduced components of electron transfer systems results in the monovalent reduction of oxygen to the superoxide radical ($O_2^{\bullet -}$). It is estimated that 2% to 4% of the oxygen consumed during normal metabolism is consumed in this way (Liu and Huang, 1996; Purvis, 2004). In addition to being formed in the mitochondria, superoxide, can also be formed in different parts of the plant cell, such as the mitochondria, the chloroplasts, the cell wall and the plasma membrane. Once formed, superoxide rapidly dismutates (either spontaneously or enzymatically) to form hydrogen peroxide (H_2O_2) and molecular oxygen. Hydrogen peroxide is relatively stable and not a particularly strong oxidant, but it can penetrate cell membranes relatively easily so it is mobile within the cell. In the presence of ferrous iron (Fe^{2+}), or other ions capable of a single electron redox change (e.g. Cu^{2+}), it can be further reduced by superoxide via the Haber-Weiss reaction to form the highly reactive and detrimental hydroxyl radical ($HO^{\bullet}$). The Haber-Weiss reaction is itself just a form of Fenton reaction, a class of reactions where reductants can facilitate the formation of hydroxyl radicals from peroxides in the presence of Fe^{2+} (etc).

3.1. The mitochondria and active oxygen species formation

The mitochondria are major consumers of oxygen, and they are also a major site of production of AOS (Purvis, 2003). So, for tissues in darkness a major source of superoxide is the mitochondrial electron transport chain. The respiratory electron transport chain, located in the inner membrane of the mitochondria of both plant and animal cells, is made up of five multiprotein complexes, and a mobile ubiquinone pool which is present in large (10-fold) molar excess compared to the other components of the electron transport chain. Electron flow from NADH to O_2 occurs through three of the protein complexes (complexes III, IV, and I) develops a

proton electrochemical gradient across the inner membrane (Figure 2). This electrochemical gradient is dissipated, (i.e., protons flow back across the mitochondrial inner membrane into the matrix), during the phosphorylation of ADP to ATP by complex V (the ATP synthase). Thus, a continuous flow of electrons through the electron transport chain to oxygen requires rapid turnover of ATP to provide ADP, and this continued flow of electrons to oxygen will serve to re-oxidize the components of the chain that are reduced by NADH. .

A restriction or disruption of electron flow through the electron transport chain, such as occurs during resting (state 4) respiration (i.e., when all available ADP has been phosphorylated to ATP) and in stressed plant tissues, results in a substantial decrease in oxygen consumption by cytochrome oxidase. As a consequence, a high concentration of oxygen builds up in the mitochondria and some of the components of the electron transport chain remain reduced. The high intra-mitochondrial oxygen concentration and high redox status of the electron transport chain favour leakage of electrons from reduced components to molecular oxygen, resulting in increased production of AOS (Skulachev, 1997). Two sites in the mitochondrial electron transport chain where single electrons can leak to molecular oxygen reducing it to superoxide are the flavoprotein components of the dehydrogenases, especially complex I, and the cytochrome bc_1 complex (complex III) (Rich and Bonner, 1978). In addition to complexes I and II, plant mitochondria have a calcium-activated NADH dehydrogenase located on the exterior surface of the inner membrane and a rotenone-insensitive NADH dehydrogenase (complex I activity is inhibited with rotenone) located on the matrix side of the inner membrane that also reduce the ubiquinone pool (Figure 2).

The plant uncoupling mitochondrial protein (PUMP) discovered by (Vercesi et al., 1995) dissipates the proton electrochemical gradient across the mitochondrial inner membrane without the production of ATP. PUMP is activated by free fatty acids, especially linoleic acid, but is inhibited by purine nucleotides (e.g., GTP). Protonated fatty acids readily cross the mitochondrial inner membrane and the anionic deprotonated fatty acids are removed by the PUMP from the matrix back into the inter membrane space resulting in fatty acid recycling and mitochondrial uncoupling (Jezek et al., 1996). As a result of this uncoupling action, mitochondrial resting potential (state 4) respiration increases with a parallel decrease in the trans-membrane electrochemical potential. In addition to cytochrome oxidase, plant mitochondria have an alternative oxidase (AOX) located on the matrix side of the inner membrane, that oxidizes ubiquinol directly without producing a proton electrochemical gradient across the inner membrane. Similar to cytochrome oxidase, the alternative oxidase reduces oxygen directly to water with four electrons. Since electron flow through the alternative oxidase is not coupled

with the synthesis of ATP, alternative oxidase activity can reduce the redox potential of the components of the electron transport chain and at the same time lower the oxygen concentration in the mitochondria (Purvis, 2004; Purvis and Shewfelt, 1993). Higher alternative oxidase activity requires reduction of the sulfhydryl groups on the enzyme and the presence of an α-keto acid, such as pyruvate, the end product of glycolysis (Umbach et al., 1994). Pyruvate interacts directly with sulfhydryl groups on the enzyme (Umbach et al., 1994), and lowers the reduced ubiquinone to total ubiquinone ratio required for maximal activity (Day et al., 1995). Recent studies with transgenic cultured tobacco cells over-expressing and under-expressing the alternative oxidase show clearly that alternative oxidase activity lowers the production of AOS when respiratory electron flow is restricted by cytochrome pathway respiratory inhibitors (Maxwell et al., 1999), and during phosphate-limited growth (Parsons et al., 1999). Thus, the alternative oxidase dissipates redox energy without building up the phosphate potential of the mitochondria.

The ubiquinone molecule can be reduced to ubiquinol by NADH derived from respiratory substrates oxidized by the TCA cycle enzymes in the mitochondrial matrix or by other dehydrogenases located in the cytoplasm. Ubiquinol is subsequently oxidized by complex III (the cytochrome bc_1 complex) with the transfer of electrons one at a time through the cytochrome path to complex IV (cytochrome oxidase), which ultimately reduces oxygen to water by the simultaneous transfer of four electrons. A relatively high proportion (almost half) of the ubiquinone, however, does not appear to be reduced/oxidized by the

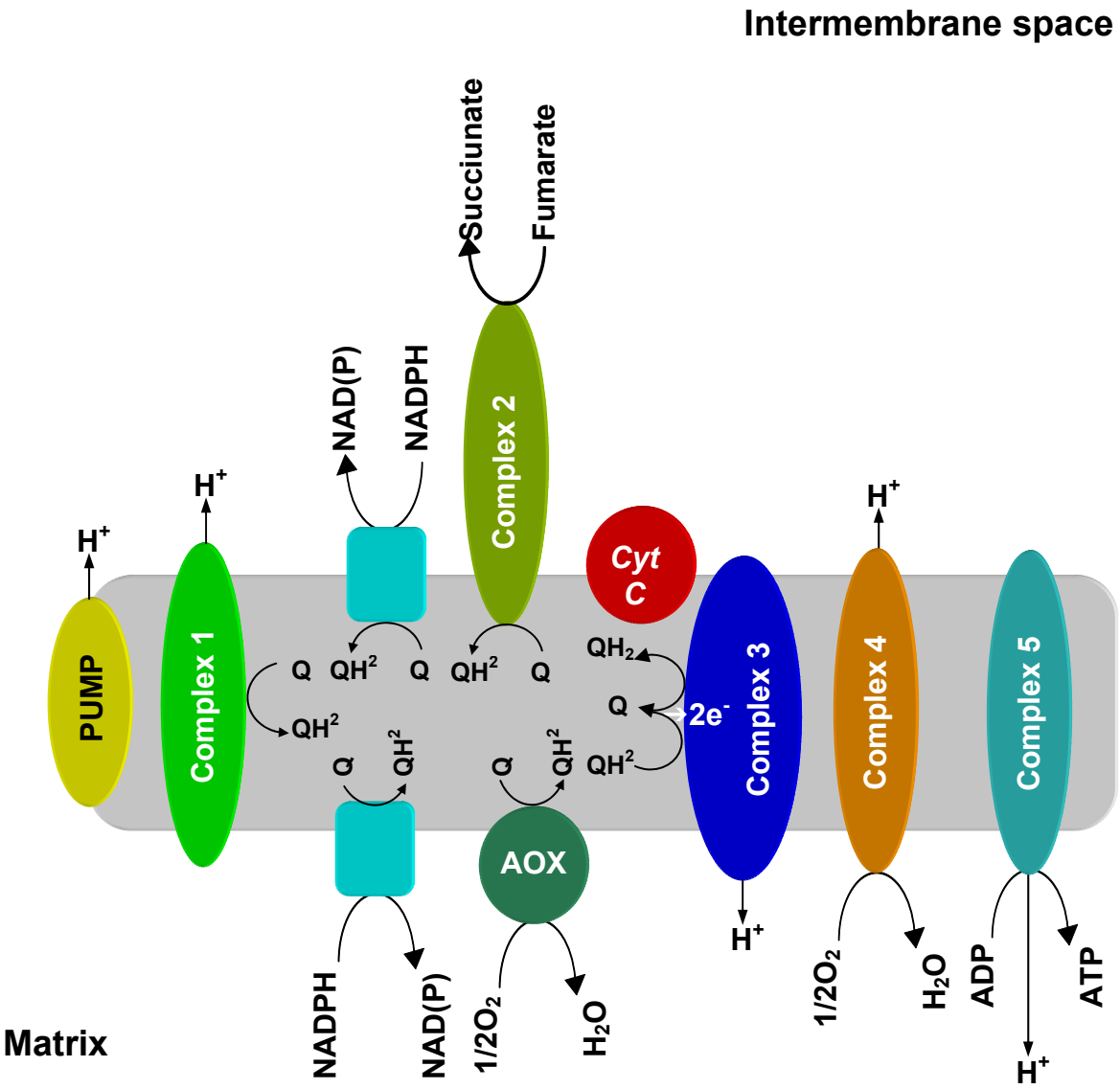


Figure 3 .The respiratory electron transport chain of the mitochondrial inner membrane in a plant cell. The five multi-protein complexes are: complex-1 (rotenone-sensitive NADH dehydrogenase), complex-2 (succinate dehydrogenase), complex-3 (cytochrome *bc*$_1$), complex-4 (cytochrome oxidase), and complex-5 (ATP synthase). The ubiquinone pool is present in large molar excess relative to the other components. Calcium (Ca^{2+}) activates an external NADH dehydrogenase, a rotenone-sensitive internal NADH dehydrogenase, a plant uncoupling mitochondrial protein (PUMP) and an alternative oxidase (AOX). (From Purvis 2004).

respiratory electron transport chain (Wagner and Krab, 1995) and may participate in the structure of the inner membrane or serve as an antioxidant (Beyer, 1992).

3.2. Chloroplast physiology as a source of active oxygen species

In chloroplasts AOS are generated from at least three sites in chloroplasts (Ulrich, 2002). Photosystem I (PSI) can reduce oxygen by Mehler reaction to form superoxide (see Scheme1) and the rate of this reaction is thought to be increased

under conditions where NADP is limiting. Photo-activated, excited chlorophyll normally transfers its excitation energy to photosynthetic reaction centers where the energy of the excited state is used to produce charge separation and then electron transport. However some of this energy, instead of producing photochemistry at the reaction centre, can excite molecular oxygen from the relatively unreactive triplet ground state to the much more reactive singlet excited state. Additionally, AOS can be formed in the photosystem II reaction centre (Telfer et al., 2003) and on the acceptor side of PSII (Villarejo et al., 2002).

Overall, however, the most important AOS generating reaction in the chloroplast is the Mehler reaction. The reaction sequence can be illustrated as follows:

$H_2O + 2O_2 \rightarrow 2O_2^- + 2H^+ + 1/2O_2$: the overall Mehler reaction

$Fd^- + O_2 \rightarrow Fd + O_2^-$: reduced ferredoxin is not the only donor

$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$,: the action of superoxide dismutase

$H_2O_2 \rightarrow H_2O + 1/2O_2$: reduction of peroxide by the ascorbate

Scheme1. The use of oxygen as electron acceptor in a Hill-type reaction by chloroplasts (Mehler, 1951).

Thus the overall electron transfer reaction results in the oxidation of water to oxygen, electron transport via PSII and PSI which will generate a trans-thylakoid proton potential, and finally the reduction of oxygen to water (scheme 1). If the superoxide detoxification reactions of the chloroplast stroma worked perfectly and destroyed all the superoxide and peroxide produced then there would be no problem. Inevitably, however, some AOS produces harmful reactions before destruction.

These reactions are, however, strictly light dependent and cannot occur in the dark conditions typical of storage. Other sources of AOS in chloroplasts are associated with metabolism rather than the 'light reactions' of photosynthesis (Robinson et al., 2004) and these could occur in darkness depending on the dark activation of oxidative metabolic pathways (e.g. the oxidative pentose phosphate pathway) in the chloroplast stroma and the fluxes of electrons to flavoproteins and FeS containing proteins. Though metabolic generation of AOS is considered to be a certainty in the light (Robinson et al 2004), its status in the dark has not been analysed. Nonetheless, it remains a possibility.

3.3. Cell wall and AOS

Various metabolic reactions which occur in the cell wall are linked to AOS formation. The most common AOS forming reactions are linked to biosynthetic

reactions, for example, the cross linking of phenylpropanoid precursors by reactions with H_2O_2 leading to random linkage of subunits to form lignin (Gross, 1980). NADH is generated via a cell wall malate dehydrogenase, which then form H_2O_2 and $O_2^{-\bullet}$ as a result of the activity of a bound Mn-peroxidase and other non-enzymatic reactions (Gross et al., 1977). Diamine oxidase is also involved in the formation of AOS in the cell wall. This enzyme uses diamines or polyamines (putrescine, spermidine, cadaverine) as substrates, and it reduces oxygen via a reduced quinone intermediate to form superoxide (Elstner, 1991; Vianello and Macri, 1991)

3.4. AOS from the plasma membrane

Superoxide-generating NAD(P)H oxidase activity has been clearly identified in plasmalemma-enriched fractions (Lesham and Kuiper, 1996). These flavoproteins may produce superoxide by the redox cycling of certain quinones or nitrogenous compounds. Toivonen (2004) has reported that low temperature stress causes a dysfunction of the NAD(P)H oxidase system in plasmalemma which leads to the formation of superoxide radical ($O_2^{\bullet-}$). Superoxide is also formed in the plasma membrane of plant cells exposed to fungal elicitors involved in activating the hypersensitive response found in some plant/elicitor responses (Doke et al., 1991; Doke and Ohash, 1988). NADH is also possibly oxidized by O_2 in the plasmalemma (Vianello and Macri, 1991).

4. Oxidative stress: sensitivity, tolerance and occurrence

As with any stress, plants normally have two primary strategies of coping with oxidative stress: they can either avoid it or tolerate it. Fruits and vegetables after harvest will rarely be in the position to actively avoid stress. Only removing the commodity from a stress will allow for the avoidance strategy. However, similar to growing plants, tolerance to oxidative stress of fruits and vegetables in the post-harvest phase has been associated with factors such as water- and lipid soluble antioxidants (Hodges and Forney, 2000; Hodges and Forney, 2003), regulation of AOS production (Duque and Arrabaça, 1999), and membrane composition (Hodges et al., 2004; Zabrouskov et al., 2002).

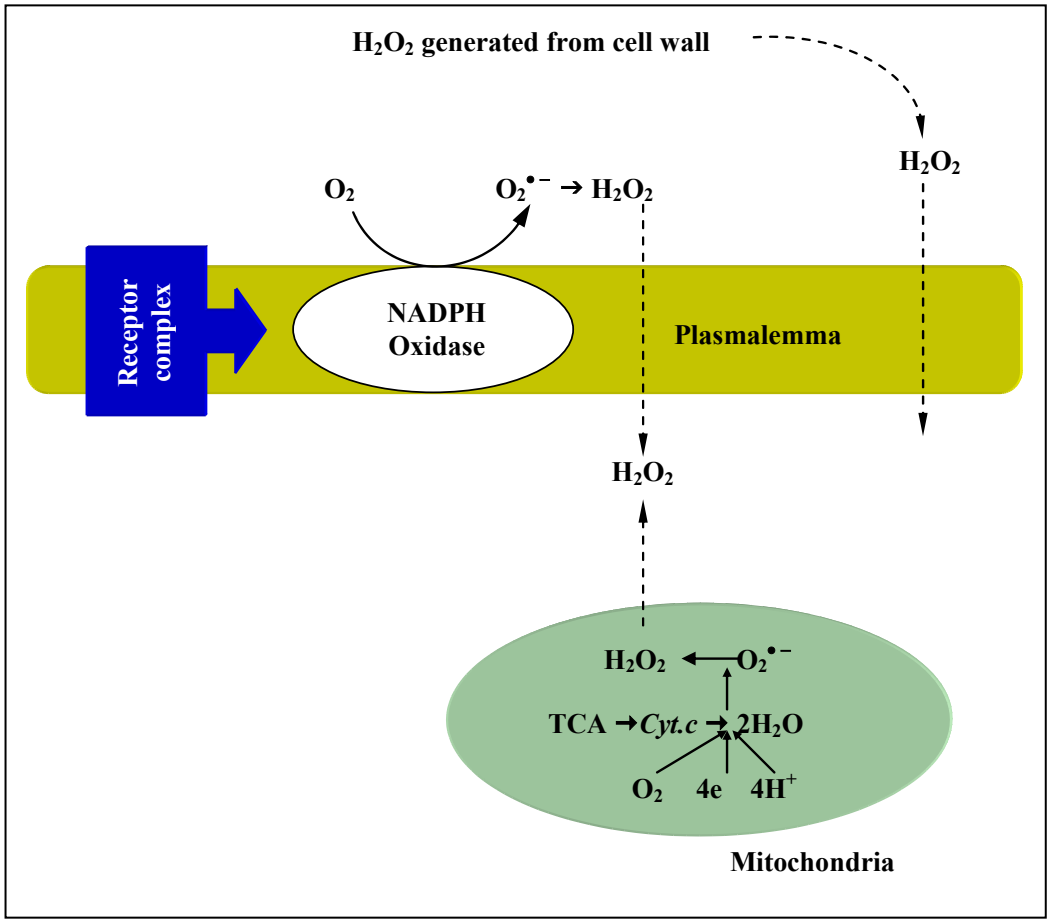


Figure 4.Reactive oxygen species produced by the plant cell at the cell membrane. This figure is based on information from (Del Río et al., 2002; Mahalingam and Federoff, 2003; Mittler, 2002) and is also described by (Toivonen, 2004). H_2O_2 = hydrogen peroxide, $O_2^{\bullet -}$ =superoxide anion, TCA = tricaroxylic acid cycle and *Cyti.c* = cytochrome c.

Factors determining sensitivity to, or occurrence of, oxidative stress

In addition to internal factors, such as antioxidant pool sizes, the physical environment of the product and its handling have a major influence on the development of oxidative stress. Below is a summary of the factors that are considered important in determining the degree of oxidative stress or injury that develops in a product post-harvest.

1. Fruit or vegetable cultivar (i.e. genotype) and harvest maturity (Gong et al., 2001; Masia, 1998).
2. Harvest procedures and storage duration (Hodges and Forney, 2000).
3. Storage temperature (Wismer, 2003).
4. Storage atmosphere (Gunes et al., 2002).
5. Processing protocols (Hodges et al., 2000; Pirker et al., 2002).
6. Conditions exacerbating water loss (Toivonen, 2003).
7. Senescence and/or ripening of the commodity (Aharoni et al., 2002; Lacan and Baccou, 1998; Lester, 2003).

4.1. Harvest maturity

Oxidative injury disorders are highly dependent upon the harvest maturity stages of the fruit or vegetable, an observation hypothesized to be related to scavenging capacities of water and lipid-soluble antioxidants such as ascorbate, or tocopherols and carotenoids, and the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) , ascorbate peroxidase (APX), and glutathione reductase (GR) (Toivonen, 2003). For example, disorders such as fruit scald have been associated with decreased antioxidant capacity (DeLong and Prange, 2003; Larrigaudiere et al., 2001). And are related to harvest maturity (Toivonen, 2003). Kader (2002) reported that the concentration of water-soluble antioxidants (such as ascorbic acid) in mango fruits depends on their maturity stage at time of harvest. It is higher in immature fruits (M1) compared with half (M2) and fully mature (M3) fruits at harvest time whereas the reverse trend was observed for the carotenoids. Barden and Bramlage, (1994) considered that the levels of lipid-soluble antioxidants during apple storage depended on maturity stage, and that the higher antioxidant content that developed at full maturity was necessary to avoid scald. Maturation of Saskatoon fruit (*Amelanchier alnifolia* Nutt.) is accompanied by increases in oxidative stress and a parallel decline in SOD and CAT activities. The anthocyanin levels in blueberry fruits increased with harvest maturity, though both the oxygen-radical absorbing capacity and phenolic content were lower in more ripe fruit (Kalt et al., 2003). These results are apparently cultivar and/or growing region dependent as another study with the blueberry cultivar 'Elliot' showed increases in antioxidant activity, total phenolic content and anthocyanin content with fruit maturity (Connor et al., 2002). Tomatoes picked at the fully ripe stage also developed higher levels of lycopene, β-carotene, and ascorbate than fruit picked stages where they are unripe (Giovanelli et al., 1999). From this it can be concluded that changes in total antioxidant content or activity of a fruit or other product during ripening, storage etc cannot be known if the changes of only a limited number of anti-oxidants are measured.

4.2. Storage temperature

Control of storage temperature is used to optimise or maintain quality attributes of fruits and vegetables, such as texture, flavour and appearance, over time. Low-temperature storage extends the shelf life of plant commodities, but product-specific temperature thresholds exist beyond which symptoms of chilling-induced oxidative injury and oxidation-related disorders will occur (Hodges et al., 2004; Toivonen, 2003; Wismer, 2003). Even the recommended optimal storage temperatures may accelerate oxidative stress and induce senescence (Zhuang et

al., 1997). The induction of oxidative stress by chilling temperatures has been well studied. In broad terms, in chilling-sensitive commodities, chilling temperatures impair the energy state of the cell, disorder metabolism, and/or provoke alterations in membrane integrity (Bartosz, 1997; Purvis, 2003; Purvis and Shewfelt, 1993). This often results in increased AOS formation, while also reducing scavenging efficacy through such factors as chilling-related inactivation of antioxidants and/or impeded antioxidant turnover (Bartosz, 1997; Wismer, 2003). Membrane lipid peroxidation may be one of the first events in the development of chilling injury (Tijskens et al, 1994). Profiles of antioxidant compounds may also be altered in response to low temperature extremes.

Sala and Lafuente (2000) found that SOD activity of the flavedo (the pigmented skin) of mandarin fruits increased in both chilling-sensitive and chilling-tolerant varieties, while CAT, ascorbate peroxidase (APX), and GR increased only in the chilling-tolerant cultivars. Purvis et al (1995) proposed that mitochondria are a source of oxidative stress during chilling as superoxide production from the mitochondrial electron transport chain is increased in chilling-sensitive green bell-peppers. A wide range of storage disorders, such as low-temperature sweetening, superficial scald, pitting, and core browning, are related to low-temperature-induced oxidative stress (Toivonen, 2003; Wismer, 2003).

An effect of chilling temperatures is to alter the homeostasis between AOS generation and defence mechanisms with the result that the net AOS burden increases and damage ensues (Hodges, 2003). The working hypothesis that arises from this simple model is that chilling-tolerant plants or plant parts may initially contain more, or can generate more, antioxidants during stress and/or produce fewer AOS than more chilling-sensitive plant species (Wismer, 2003).

4.3. Water status

Hodges et al (2004) reported that there was a relationship between water loss during the post-harvest storage and AOS generation, and high humidity during storage has been shown to reduce chilling injury (Forney and Lipton, 1990). Moreover under non-chilling conditions, wilting has been associated with decreases in antioxidants, such as ascorbic acid, in cabbage and snap bean (Toivonen, 2003). Water loss has been shown to cause an increase in the production of AOS, especially H_2O_2, in plant tissues (Yan et al., 2003). For example, in muskmelon (Lester and Bruton, 1986) and mango fruits (Pesis et al., 2000) it has been found that a high humidity achieved by MAP or a plastic film package with a selective gas permeability prevented the development of chilling injury and prevented the loss of carotene, fruit sugars, non-enzymatic browning,

membrane integrity and fresh weight, while maintaining fruits firmness and marketable appearance. In retail displays, using water mists at non-refrigerated temperatures reduces both tissue water loss and promotes the retention of ascorbic acid and total carotene in muskmelon fruits (Barth and Zhuang, 1996).

4.4. Processing

Physical abrasions, high or low processing temperatures, and product preservation techniques may all induce an increase oxidative stress (Hodges et al., 2000). For example, Pirker et al. (2002) stated that the AOS content of freeze-dried strawberry samples was approximately 10 times higher than that of frozen strawberries samples. In a study comparing air and MAP storage, followed by cooking in water, on the ascorbate content of fresh-cut spinach, a decrease in the total antioxidant activity was observed during 7 days of storage, but with ascorbate levels remaining higher in MAP atmospheres (Gil et al., 1999). Subsequent boiling of the spinach in water for 10 min resulted in a 60% loss of ascorbate to the surrounding water.

Irradiation with ionizing radiation, such as gamma rays or electron beams, induces the development of AOS that can lead to oxidative disorders in both fruit and vegetable tissues (Toivonen, 2003). Ionizing irradiation produces ozone both in air and any in oxygen-containing plant tissues, especially in air-filled apoplastic spaces (Maxie and Kader, 1966). These increases in AOS are in addition to the primary damage that ionizing radiation causes to plant membranes, proteins and DNA (Jones and Bulford, 1990).

Various disorders due to post-harvest irradiation of products have been reported by Hodges et al (2004) and many involve decreases in anti-oxidative capacity. Reported problems include: lower sensitivity to ethylene in apples (Abeles, 1973), increased electrolyte leakage (Lester and Wolfenbarger, 1990) and decreases in the ascorbic acid (Garcia-Yanez et al., 1990.) and glutathione (Toyo'oka et al., 1989) levels in grapefruit, de-esterification of phospholipids in cauliflower (Voisine et al., 1991), and losses of carotenoids in potatoes (Mitchell et al., 1990). Lester and Wolfenbarger (1990) found that a 0.25 kGy dose greatly increased measurable membrane damage (electrolyte leakage), loss of total phenols and ascorbic acid levels in fresh grapefruit**.** However although there are many examples of oxidative disorders developing due to ionizing irradiation, the decline in marketable quality and nutritional value may be contained within acceptable limits if appropriate doses/rates are applied (Toivonen, 2003). (Kader, 1986) has summarized the recommended safe doses of ionizing irradiation for fresh fruits and vegetables.

Ultra-violet irradiation in the UV-B and UV-C bands can also can affect AOS levels in plant tissues. Schmitz-Eiberger and Noga (2001) found significant changes in green bean seedling leaves following UV-B (280-320 nm) irradiation. UV-B-induced injury resulted in a disturbance of the active oxygen metabolism system by destroying non-enzymatic antioxidant mechanisms (for example the ASC pool) and altering the enzymatic antioxidant defence systems (for example SOD and APX activity decreased). Barka (2001) showed that immediately upon exposing tomato fruit to a low dose of UV-C irradiation (3.7 $kJ \cdot m^{-2}$ at 254 nm), superoxide dismutase and ascorbate oxidase activities were decreased while those of lipoxygenase and phenylalanine ammonia lyase were increased. Much is still unknown about the development of oxidative stress in fresh fruits and vegetables in relation to energetic irradiation of various kinds, and this is, therefore, still an important area for research (Hodges et al., 2004).

4.6. Ripening and AOS

Ripening is associated with rapid changes in cellular components, an increase in catabolic processes, and the initiation of senescence (Masia, 2003). So, ripening, which involves developmental changes in color, firmness, sugar, flavor, and proteins, can be differentiated from senescence as the latter involves a loss of membrane integrity and tissue death (Noodén, 1988). During ripening, levels of AOS progressively increase, due mainly to the declining activity of antioxidant enzymes that diminish AOS levels. Aharoni et al. (2002) conducted a comprehensive investigation of gene expression during the maturation and non-climacteric ripening of strawberry. Gene expression of ripening fruits was compared to gene expression in the fruits subjected to oxidative stress following their treatment with the free-radical generator 2,2'-azobis(2-propanimidamide) dihydrochloride. It was found that of the 46 genes that were induced by oxidative stress, 20 genes were also upregulated during ripening, and of these some were clearly linked to anti-oxidative activity (e.g. ferritin, glutaredoxin, glutathione-S-transferase)

Rogiers et al (1998) clearly showed that the ripening of climacteric Saskatoon fruit (*Amelanchier alnifolia* Nutt.) is associated with a rise in AOS that affects membrane lipids. The increase in lipid peroxidation during fruit ripening was suggested by the evolution of ethane and the development of thiobarbituric acid-reactive substances (TBARS), both of which are indicators of the breakdown of oxidised membrane lipids. At the same time the activities of SOD and CAT enzymes declined 4-fold and 18-fold, respectively, and under these circumstances cytotoxic levels of H_2O_2 might accumulate. Lipoxygenase activity increased 2.5-fold during ripening, while the reduced and oxidized forms of glutathione increased

during the later stages of ripening with only a minimum (3%) variation of the percentage of GSSG relative to the total of (GSG+GSSG). In ripening pepper fruits, higher ascorbate peroxidase and Mn-SOD activities in the mitochondria play a role in avoiding the accumulation of AOS (Jiménez et al., 2003). The presence of elevated levels of the antioxidant enzyme system implies that the management of oxidative stress is a key factor during fruit and vegetable ripening.

4.5. Senescence

Senescence is considered the terminal phase of life of plant organs, including leaves, flowers and fruits (Buchanan-Wollaston, 1997). The most intensively investigated plant tissue regarding senescence and AOS are those of leaves. In senescing leaf systems lipid peroxidation is typically associated with a decline in catalase and superoxide dismutase activities, increased levels of H_2O_2 and reduced transcription of mRNA (Watkins and Rao, 2003). Few studies have attempted to identify differences in AOS activities in relation to different rates of senescence among fruits and vegetables (Hodges et al., 2001). (Du and Bramlage, 1994) found that total SOD activity (i.e., CuZn-SOD, Fe-SOD and Mn-SOD) changed greatly during senescence of various apple cultivars, while (Jiménez et al., 2003), comparing green versus red peppers (*Capsicum annuum*), found that numerous antioxidants play a role in pepper senescence. Superoxide diumutase isozymes (Mn-SOD, Fe-SOD and CuZn-SOD), GR, CAT and APX exhibited higher activity levels in the red peppers, which were more senescent, than in the green fruits, which were less senescent. In contrast, however, monodehydroascorbate reductase and dehydroascorbate reductase activities were higher in the green fruit. These correlations imply that SODs, CAT and APX are probably involved in pepper fruit senescence.

4.7. Ethylene

The processes of ripening and senescence in fruits and vegetables are associated with oxidative mechanisms (Masia, 2003). Ethylene is commonly considered as the hormone that regulates ripening, and exposure of sensitive plant tissues to this phytohormone results in acceleration of both ripening and/or senescence (Hodges and Forney, 2000). However, oxidative processes, such as lipid peroxidation, which are induced in some fruits in response to ethylene exposure (Meir et al., 1991), appear to be secondary effects and not as a direct result of ethylene exposure (Toivenen, 2003). In addition, many commodities are insensitive to ethylene and in these cases the processes of ripening and senescence are not accelerated by exposure to the hormone (Palou et al., 2003).

Ethylene synthesis, on the other hand, is enhanced by environmental and biological stresses such as wounding or pathogen attack (Morgan and Drew, 1997; Ohtsubo et al., 1999) and it may be stimulated by AOS and lipoxygenase activity during membrane lipid peroxidation, further accelerating ripening and senescence processes (Hodges, 2003).

5. Oxidative reactions in stressed tissues

Oxidative stress occurs when the generation of active oxygen species (AOS) exceeds the capacity of the plant to maintain cellular redox homeostasis, or, more simply, when the production of AOS exceeds the capacity of the plant to scavenge them. Lipids, proteins, carbohydrates, and nucleic acids are all targets of AOS (Hodges, 2003). Chloroplasts, mitochondria, nuclei, glyoxysomes, and peroxisomes all represent major sites of AOS production, though the importance of chloroplastidic sources is likely to decrease in ripening fruits and commodities which are usually stored in dark conditions. Membranes contain unsaturated fatty acid residues which are particularly vulnerable to oxidation, so oxidative damage to membranes is of particular importance.

5.1. Lipid peroxidation

The lipid bilayer membrane is composed of a mixture of phospholipids and glycolipids in which the fatty acid chains are attached to the C1 and C2 of the glycerol backbone by ester linkages. The peroxidation reactions are different among different fatty acids and depend upon the number and position of double bonds in the acyl chain. Lipid peroxidation reactions involve the three distinct steps typical of free-radical chain reactions: initiation, propagation, and termination (Franke, 1985). In the initiation step an AOS, such as a hydroxyl radical, reacts with an unsaturated fatty acid residue (e.g. Linoleate) in the hydrophobic core of the membrane abstracting an H atom and forming a methylvinyl radical on the fatty acid chain:

$OH^{\bullet} + RH \rightarrow R^{\bullet} + H_2O$

This and subsequent reactions are also illustrated in figure 4; in this case a hydroxy radical reacts with carbon number 11 of the linoleate residue to form a radical which is partly stabilized by being delocalized across the two conjugated double bonds that now exist between carbon atoms 9 to 13 (Figure 5). During the propagation reaction, this free-radical reacts with normal ground state triplet oxygen, which is a biradical, to form a peroxy radical:

$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$.

In the case of linoleate when this reaction occurs on carbon atoms 9 or 13 the peroxy radical formed abstracts a hydrogen atom from another fatty acid molecule to form a hydroperoxide and creating another vinyl radical on the attacked lipid. This can then react with oxygen (as before), or abstract a hydrogen from another lipid which though eliminating the first radical, creates another radical which can react with oxygen etc:

$ROO^{\bullet} + RH \rightarrow R^{\bullet} + ROOH$

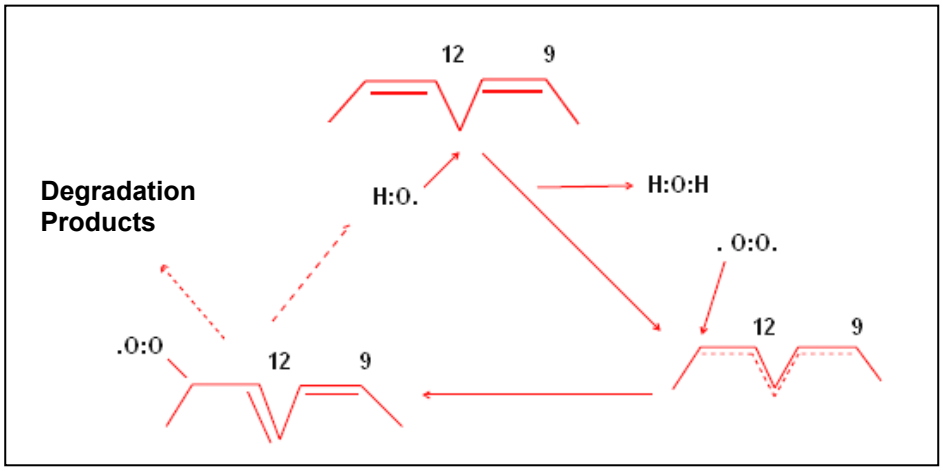


Figure 5. The peroxidation of linoleic acid. The hydroxyl radical abstracts an H atom from carbon-11 of the fatty acid between the two double bonds and is thus converted to water. The free radical that is produced on the fatty acid is delocalized between carbons 9 to 13 and is thus to some degree stabilised. Triplet molecular oxygen (the ground state that has two unpaired electrons and may attack this structure at either carbon -9 or -13 forming a alkyl-peroxy radical. This peroxy radical can then react in various ways, for example by abstracting another hydrogen atom from a second linoleic acid molecule in a propagation reaction forming a lipid hydroperoxide as shown in the diagram. The various peroxo and other oxo-adducts that are formed are unstable and various bearkdown reactions, such as chain breakage and cross-linkage reactions subsequently occur to produce aldehydes, hydrocarbons, alcohols, and cross-linked dimers.

The role of initial hydroxy radical is to start an oxidative reaction chain reaction that forms unstable, reactive lipid peroxides and free-radicals. The peroxidised lipid chains disrupt membrane structure owing to their hydrophilic character and they may spontaneously break down forming a wide range of products, some of which are toxic or reactive. Typical lipid peroxidation degradation products of ROOH are aldehydes, such as malondialdehyde, and hydrocarbons, such as ethane and ethylene. The termination reaction, which stops the free-radical chain reaction, can be a reaction between two lipid free-radicals, or a reaction between a free-radical and a membrane anti-oxidant such as tocopherol (Baier and Dietz, 1999)

5.2. Protein oxidation

During the oxidation of proteins, oxidation starts on the hydrocarbon portion of protein molecule in the vicinity of amino acid group, and this leads to amino acid modification, followed possibly by fragmentation of the peptide chain. Protein oxidation reactions are initiated by the attack of $^{\bullet}OH$ and abstraction of α-H atom of amino acid to form a carbon-centred radical. This can abstract a hydrogen from a nearby group thus forming a new radical, which may react and cross-link with an adjacent organic radical, or it may react with molecular oxygen to form an alkylperoxyl radical. This radical can be reduced to an alkylperoxide by superoxide in the presence of a ferrous ion (or similar) catalyst and the peroxide formed may then be cleaved to form an alkoxyl radical again by superoxide in the presence of a ferrous ion catalyst. Finally, the alkoxyl radical can be reduced to an alcohol by reduction by superoxide in the presence of a ferrous ion catalyst. Though reduction to an alcohol is one possibility, other reactions can occur that result in peptide chain crosslinkage, cleavage, or reactions with other molecules, such as sugars or lipids. One of the most important products of the oxidative reactions are the protein carbonyl derivatives. These are produced by a range of reactions, for example by the cleavage of peptide chain by the α-amidation pathway or by the oxidation of glutamyl side chain leading to the formation of NH_3 and free α-ketocarboxylic acid. (Berlett and Stadtman, 1997). Oxidation of lysine, arginine, proline, and threonine, will also yield carbonyl derivatives. In addition, carbonyl groups may be introduced into the peptide chain by reactions with the beakdown products of peroxidized lipids, such as the aldehydes 4-Hydroxy-2-nonenal and malondialdehyde (Schuenstein and Esterbauer, 1979; Uchida and Stadtman, 1993). The presence of carbonyl groups in proteins has been widely used an indicator of AOS linked oxidation of cell constituents, though the diverse routes by which these carbonyl groups are formed can complicate simple interpretation of the results. Several sensitive methods for the detection and quantition of protein carbonyl group have been developed (Levine et al., 1994).

6. Postharvest oxidative disorders

The classical post-harvest disorders associated with oxidative damage disorder**,** as documented by Purvis (2004), include superficial scald (DeLong and Prange, 2003) and core browning (Larrigaudiere et al., 2001), bleaching of pigments (Elstner and Osswald, 1994) and disruptions in membrane integrity (Biedinger et al., 1990). The inactivation of many types of functional proteins (e.g., enzymes) resulting from damage is followed by increased protease activity

(Casano et al., 1994; Landry and Pell, 1993), and lesions and damage to nucleic acids are also common postharvest- disorders associated with oxidative stress. Active oxygen species have also been implicated in the regulation, properties, and/or dynamics of induced or natural senescent processes (Droillard et al., 1987; Hodges and Forney, 2003; Philosoph-Hadas et al., 1994; Thompson et al., 1991). A major characteristic of senescence in plant tissues is increased lipid peroxidation (Kunert and Ederer, 1985; Lacan and Baccou, 1998). Lipid peroxidation is thought to play an important role in the biosynthesis of ethylene (Paulin et al., 1986), a hormone involved in the regulation of senescence.. Although the exact mechanism of ethylene synthesis under these circumstances is unclear, but it may arise as a lipid-fragmentation product through following the oxidation of unsaturated centres in the acyl chains of fatty acids by lipoxygenase (Gardner and Newton, 1987). This enzyme catalyzes the production of hydroperoxy conjugated dienes from polyunsaturated fatty acids.

7. Defence mechanism against AOS

In addition to causing damage to a range of cell components, AOS also serve as signals or messengers that trigger an increase in the production of antioxidants and active oxygen scavenging enzymes (Vranová et al., 2002). They are also involved in programmed cell death, which they can modulate in two ways. They can act as signalling molecules to initiate cell death or they can directly kill the plant cell (Fath et al., 2002). They are also involved with the senescence of plant tissues (Rubinstein, 2000). The level of AOS in plant tissues must therefore be regulated. This regulation is achieved by a variety of mechanisms, which includes an elaborate scavenging system that depends upon active oxygen scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and the various peroxidases, and a range of lipid-soluble (α-tocopherol and the carotenoids) and water-soluble (ascorbic acid, glutathione and the flavonoids) antioxidants (Noctor and Foyer, 1998).

7.1. Water-soluble antioxidants.

Ascorbic acid is an important quality component of many fruits. It is also an essential constituent of the human diet. Apart from preventing the well-known vitamin C deficiency disease scurvy, this substance has gained increasing recognition as playing a diverse role in maintaining human health. It is now generally accepted that vitamin C is one of the most important free radical scavengers in plants, animals and humans (MacKersie and Lesham, 1994). In

plants, ASC is found in the chloroplast, cytosol, vacuole and extra-cellular compartments (Foyer and Halliwell, 1976). Ascorbic 1acid can directly and indirectly scavenge AOS (Figure 6) with or without the involvement of enzyme catalysts, and indirectly by recycling oxidized tocopherol to the reduced form (Foyer, 1993). It reacts with superoxide, hydrogen peroxide or the tocopheroxyl radical to form monodehydroascorbate and/or didehydroascorbate. These oxidised forms can be recycled back to ascorbic acid by means of monodehydroascorbate reductase and dehydroascorbate reductase using reducing equivalents from NADPH and glutathione, or the dehydroascorbate may decompose into tartaric and oxalic acid (Figure 7 and 8) (Foyer 1993). Furthermore, ASC is thought to protect α-tocopherol (α-TOC) and recycle α-tocopheroxyl radical as shown in figure 9 (Munné-Bosch and Alegre, 2002; Smirnoff and Wheeler, 2000).

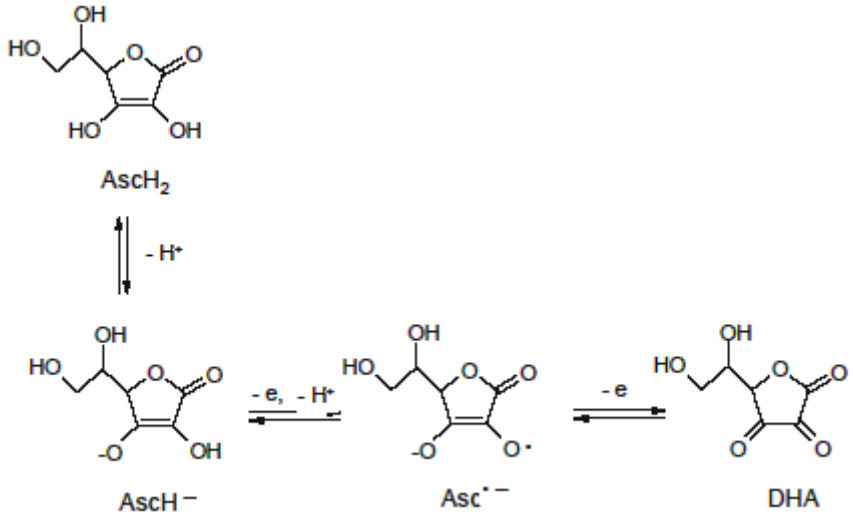


Figure 6. Oxidation of ascorbic acid to dehydroascorbic acid, modified from (Bors and Buettner, 1997; Niki, 1991)

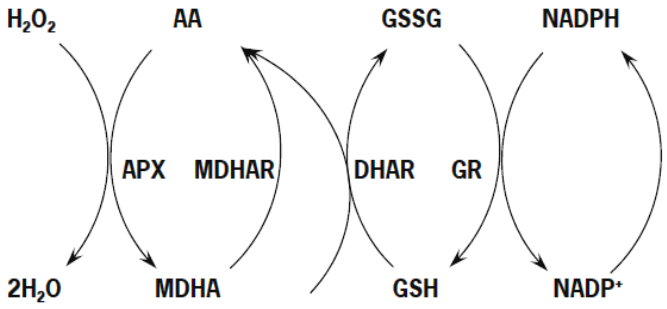


Figure 7. Halliwell-Asada pathway or ascorbate-glutathione cycle: APX, ascorbate-peroxidase; MDHAR, onodehydroascorbate reductase; DHAR, dehydroascorbate reductase; GR, glutathione reductase. From (May et al., 1998).

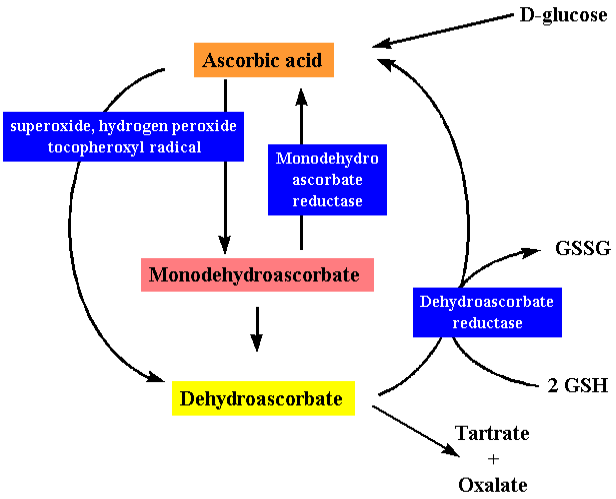
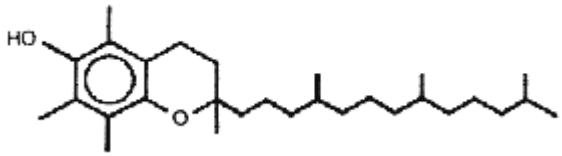


Figure 8. The degradation of L-ascorbic acid in plant tissues

7.2. Lipid-soluble antioxidants

7.2.1. α-Tocopherol (α-TOC)



α-Tocopherol acts to chemically stabilize the inner regions of the membrane bilayer. It achieves this feat owing to its properties as an antioxidant which can chemically and physically scavenge a range of AOS, such as oxygen free radicals, lipid peroxyl radicals and singlet oxygen (Diplock et al., 1989). α-Tocopherol synthesis occurs in plastids, including chloroplasts (Bramley et al., 2000). The aromatic ring is formed by the shikimic acid pathway and the phytyl chain is synthesized from the geranylgeranyl pyrophosphate and terpenoid pathways. The antioxidant properties of α-tocopherol are based upon its ability to quench both singlet oxygen and peroxide, though it is a less efficient scavenger for $^1O_2$ than β-carotene**.** It is also able to react with the peroxyl radical formed by the addition of O_2 to the vinyl radical formed on the fatty-acid tails of membrane lipids. This reaction terminates the radical chain reaction because even though a tocopheroxyl radical is the product of the reaction, this radical is stable and does not react further. As a result, tocopherol is considered an effective free radical trap. An important feature of the tocopheroxyl radical is that the radical is stabilised by being

delocalised on the phenol ring (Figure 9), which, owing to its hydroxyl group, is also the most hydrophilic part of the molecule. It is this part that will be near the surface of the bilayer, which is also where the hydrophilic peroxyl radicals will be found and where the tocopheroxyl radical can be most easily reduced by water-soluble ascorbate (Foyer, 1992).

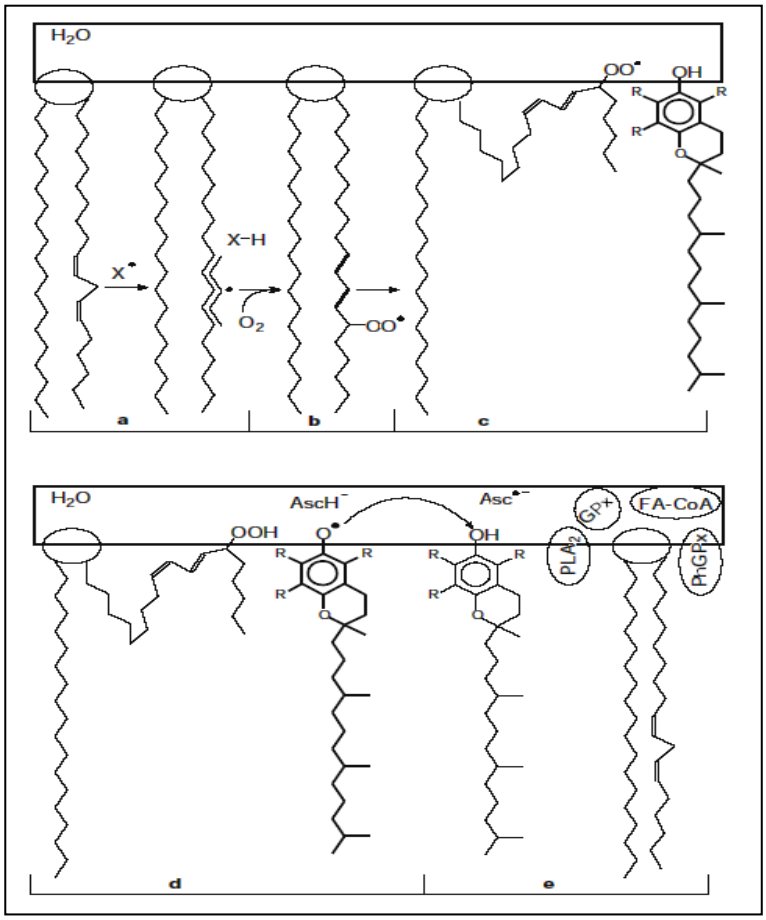
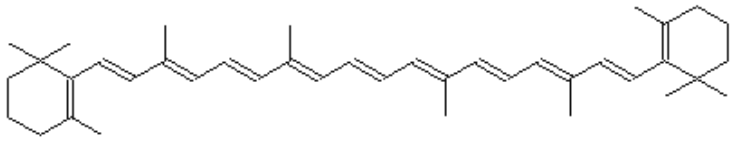


Figure 9. The formation of the α-tocopheroxyl free radical and its subsequent reduction to tocopherol: (a) Initiation of the peroxidation reaction when a free-radical X•, abstracts a hydrogen atom from an unsaturated acyl chain forming a linear pentadienyl radical.(b) this is then oxygenated to form a peroxyl radical and a conjugated diene. (c) The hydrophilic peroxyl radical moiety moves to the water-membrane interface where it available for repair by tocopherol. (d) the peroxyl radical is reduced to a lipid hydroperoxide, and the tocopherol oxidized to a tocopheroxyl radical, which can then be reduced by ascorbate. (e) Tocopherol has been recycled by ascorbate; the resulting ascorbate radical can be recycled by enzyme systems. A range of enzymes including phospholipase A2 (PLA2), phospholipid hydroperoxide glutathione peroxidase (PH-GPx), glutathione peroxidase (GPx) and fatty acylcoenzyme A (FA-CoA) may then reduced and repair the oxidized acyl chain of the phospholipid (Buettner and Schafer, 1999).

7.2.2. β-Carotene (CAR)



Carotenoids are C40 isoprenoids and tetraterpenes that are located in plastids of photosynthetic and non-photosynthetic plant tissues. In chloroplasts, one function of carotenoids is to act as an accessory pigment in light harvesting. Another important role in chloroplasts is the to detoxify AOS and convert triplet chlorophyll produced during photosynthetic light-harvesting to the singlet ground state Triplet chlorophyll is not harmful, but it is potentially relatively long-lived and thus it could react with triplet ground state molecular oxygen to form the highly reactive singlet excited state (Hideg et al., 1998). $^3Chl^*$ can transfer its excitation energy and triplet character to adjacent carotenoid molecules to form triplet carotenoids and singlet ground state chlorophyll. The triplet carotene then decays very rapidly to the ground state, thus eliminating the triplet character of the molecule and preventing singlet oxygen formation:

$^3Chl^* + {}^1\beta\text{-car} \rightarrow {}^1Chl + {}^3\beta\text{-car}^*$

$^3\beta\text{-car}^* \rightarrow {}^1\beta\text{-car} + \text{heat}$

Another carotenoid, zeathanthin plays a particularly important role in the regulation of photosystem II. This xanthophylls acts in a way that it is not yet fully understood to facilitate the de-excitation of excited singlet chlorophyll to the ground state, with the release of the excitation energy as heat. This quenching mechanism competes with the reaction centres of photosystem II as quenching route, and thus reduces the likelihood that charge separation will occur in the reaction centre. This results in a reduction in the quantum efficiency of electron transport by photosystem II. Under light-limiting conditions this loss of efficiency would result in a loss of CO_2 fixation capacity, but at higher light intensities this loss of quantum efficiency seems to act to protect photosystem II reaction centres from photoinhibition, a process which also seems to involve active oxygen.

7.3. Antioxidant enzymes

The continuous oxidative stress that cells are under results in their having a comprehensive protection system to guard against injury from AOS. The extent to which this protective system increase its activity in response to an oxidative challenge, or the extent to which the increase in oxidative damage correlates with decreases in the activity of certain protective mechanisms is valuable as *a priori* evidence of possible regulation of control systems and causal pathways leading to

injury. Plants have both enzymatic and non-enzymatic antioxidant systems to prevent or alleviate the damage from AOS. Many enzymes can efficiently reverse or detoxify the effects of AOS (e.g. lipid hydroperoxidases (Baier and Dietz, 1999), whereas others act to detoxify various AOS before they can cause damage, for example $O_2^{\bullet-}$ is detoxified by SOD, and H_2O_2 is removed by CAT and by reactions catalysed by different kinds of peroxidases (e.g. guaiacol peroxidases (GPX). A major H_2O_2 detoxifying system in plants is the ascorbate-glutathione cycle that includes APX and GR (Asada, 1994). These protection systems are not perfect, however, and an increase in the production of AOS or a decrease in scavenging capacity will result in an increase in oxidative damage. This will first produce an increase in lipid and protein oxidation followed by the development of classical, oxidative postharvest disorders (Hodges et al., 2004).

Generally, however, plants show a rapid response to increased AOS produced by physical stresses (in response to, for example, changes in storage conditions e.g. temperature and time (Rogiers et al., 1998). These changes lead to an enhancement in cellular oxidants that induce an increase in the activities of non-enzymatic antioxidants such as ASC, α-TOC and β-CAR, and antioxidant enzymes activity such as SOD CAT, GR, and APX (Bowler et al., 1992; Foyer and Harbinson, 1994). The effects of storage conditions on the actions of the natural antioxidants are studied in details in chapter 4 and 5. (Saltveit and Morris, 1990) have reported that many fruits have differing sensitivities towards chilling temperatures depending on their maturity stage or stage of development once fully mature. Studies on the chilling sensitivity of pre and post-climacteric fruits have shown that pre-climacteric Avocado, papaya and mango fruits are more sensitive to low storage temperatures (Robert, 1990).

8. Increasing chilling tolerance chilling injury

Chilling sensitivity is observed in many plants or parts of plants whose vitality or post-harvest longevity are diminished by exposing the them to temperatures from 0^{o}C (non-freezing) to about 15^{o}C (Wang, 1990). A range of processes have been suggested to be the causal factor differentiating chilling tolerant from chilling sensitive tissues, cultivars or species. There are also many different studies investigating the genetic basis for this physiological phenomenon and exploring its variability. Genetically, variability for chilling tolerance exists in most crop species, such as tomato (Walker et al., 1990) and peanut (Bell, 1993). Many breeding programs have attempted to transfer the genes of chilling tolerance from tolerant plants into sensitive plants, but this is often a complex and time consuming process. Chilling tolerance is usually found in distant wild plant relatives of

commercial plants. When those plants were used in plant breeding programs to increase the tolerance of the sensitive plant there was also loss of commercial quality. Breeding for improved or heightened oxidative stress defense in cucumber fruits by increasing the CuZn-SOD levels was recently accomplished by transgenic manipulation (Lee et al., 2003).

An alternative approach is to exploit the cross-tolerance phenomenon were increasing a plant's resistant to one stress increases its resistance to others. For example heat stress will provoke physiological responses that also increases the chilling resistance. Heat treatment of mangoes in air or water will reduce chilling injury symptoms and increase their shelf-life, but these treatments have a negative effect on mango quality and are therefore unusable in practice (Majeed and Jeffery, 2002).

9. Methods for the assessment chilling injury

In addition to assessing the degree of chilling injury by means of scoring the visual symptoms of injury (Walker et al., 1990) a range of other techniques are available with which to assess damage.

9.1. Chlorophyll fluorescence

One of the most sensitive responses to low storage temperatures is damage to photosystem II. This may be measured by means of the non-destructive chlorophyll fluorescence (CF) technique. It is important to note that damage to photosystem II can be produced not only by exposure of chilled tissue to a relatively high irradiance, but also by storing tissue in darkness under chilling conditions. The damage to photosystem II provoked by the combination of light and chilling is a well studied phenomena called photoinhibition, and it is probably a consequence of photochemical activity in the photosystem II reaction centre resulting in singlet oxygen formation rather than electron transport. Damage to photosystem II provoked by low temperatures in darkness develops under more severe conditions (lower temperatures, longer duration of chilling) and its physiological basis is not known, though clearly it cannot involve photochemically induced injury as light is absent. Phase transitions in the thylakoid membrane have been associated with the dark-induced damage to photosystem II activity temperature that coincide with temperature of the phase transition in the polar lipids of thylakoid membrane (Meir et al., 1995; Parkin et al., 1989). Damage to photosystem II, measured by means of chlorophyll fluorescence, is both a very sensitive and convenient indicator of damage to the thylakoid membrane, as shown in apple (DeEll, 1996) and cucumber (Tijskens et al., 1994). The chlorophyll

fluorescence parameter used to assess damage to photosystem II is the F_v/F_m ratio, F_m is the maximum relative fluorescence yield produced in dark-adapted material subjected to an irradiance sufficient to reduce all the Q_A pool, F_v is F_m minus F_0, where F_0 is relative fluorescence yield measured from dark-adapted material when the Q_A pool is oxidised (i.e. non-reduced). An F_v/F_m ratio near 0.8 is typical of healthy, unstressed material (Björkman and Demmig, 1987; Bolhàr-Nordenkampf et al., 1989). Reductions in Fv/Fm below this value are widely considered as being indicative that the material has been stressed, though often the causal relationship between the stress and the reduction in Fv/Fm is not explored or explained.

Because of its convenience, the CF technique has been explored as a possible tool for the quantitative assessment of post-harvest chilling injury produced during storage: it is rapid, sensitive, non-destructive to tissues and able to detect injury before visible symptoms appear (DeEll, 1996; MacRae et al., 1986; Schreiber and Bilger, 1987). Different applications of CF in the post-harvest phase during storage under low temperatures have been used to evaluate chilling injury development in many fruits; for example banana (*Musa acuminata* Colla) and mango (*Mangifera indica* L.) (Smillie et al., 1987), in cucumber (van Kooten et al., 1992) and green pepper (*Capsicum annuum* L.) (Lurie et al., 1994).

9.2. Ion leakage

Ion leakage is a widely used technique for measuring damage to tissues, especially following low temperatures stresses, such as chilling or freezing, applied to sensitive plants. Ion leakage requires that the cell membrane looses its semipermeable nature, but whether this is a primary or secondary effect of the stress depends on the mode of action of the stress. The two syndromes that could lead to leakage as a result of chilling injury are summarized below (Hariyadi and Parkin, 1991), in one case injury to the cell is a primary effect, in the other it is secondary:

1) Liquid-crystal to gel phase transitions in lipid membranes, possibly due to gel-phase separation of high melting point phospholipids. This process has been proposed to lead to the development of chilling injury in sensitive plants and their organs. Gel phase regions are more fragile, their interfaces with the liquid crystal zones are leaky, and the remaining liquid crystal zones may be unstable. All of these will lead to leakiness

2) Low storage temperatures induce dysfunction of enzymes and a redistribution of cellular calcium. This leads to metabolic changes that

increase the net formation of AOS. These will damage cell membranes by oxidizing them. Oxidized membranes are more brittle, and the introduction of hydrophilic peroxy groups into the hydrophobic core of the membrane disrupts the bilayer structure, which is responsible for the impermeability of the membrane. The gel phase transition temperature also increases as the membrane oxidizes – see point one.

In general, an increase in ion leakage is considered to be good evidence for tissue injury, such as that induced by chilling. It is a simple technique and very widely employed.

Chapter 3

Chlorophyll fluorescence assessment of chilling injury in 'Zibdia' and 'Hindi Be-Sennara' mango varieties

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Abstract

Chlorophyll fluorescence (F_v/F_m ratio) of mango (*Mangifera indica* L.) fruits was evaluated as an indicator of chilling injury development during storage. The F_v/F_m ratio of two mango varieties 'Zibdia' and 'Hindi' was determined during a 36 day storage period at temperatures of 2, 4, 7, 10 and 20^oC and at different harvest maturity stages, i.e. immature (M1), half-mature (M2) and fully mature (M3). In general, 'Zibdia' mango had a higher incidence of chilling injury at low temperatures than 'Hindi', and the M3 fruits revealed more injury than M1 and M2 fruits in both mango varieties. However, the initial occurrence of injury after 10 days of storage was similar for both varieties and it was only after 20 days of storage that it was possible to identify differences between the cultivars. The F_v/F_m ratio of dark-adapted fruits decreased rapidly after 12 days of storage in 'Zibdia'. In 'Hindi' it decreased gradually until the end of the experiment. However, harvest maturity stages did not present any differences in F_v/F_m ratio between both varieties. The correlation of the F_v/F_m ratio with chilling injury was observed for both varieties during storage. The results of this study suggest that F_v/F_m ratio during storage may be a good tool to assess chilling injury development before visible symptoms appear. Zibdia is more sensitive to low storage temperatures than the Hindi cultivar and the sensitivity varied according to the harvest maturity stages.

Keywords: Chilling injury (CI); Chlorophyll fluorescence (CF); F_v/F_m ratio; Storage; Mango; *Mangifera indica* L.

1. Introduction

Mangoes, like many other tropical and subtropical fruits, are susceptible to chilling injury (CI) when stored at low temperatures above freezing point. In the case of mango chilling injury begins at temperatures below 12^{o}C (Chaplin et al., 1991; González-Aguilar et al., 2000). The most common visual symptoms of CI in mango fruits are dark skin, a scald-like discoloration, and pitting or sunken lesions on the peel (Chaplin et al., 1991; Nair et al., 2003); an abnormal ripening of the fruit and followed by decay is another consequence of storing mangoes at temperatures below 12^{o}C. The visible symptoms associated with chilling injury depends upon the plant species, the degree of tissue maturity, and the storage period and temperature during storage (Wang, 1990). These visible symptoms are the result of the disruption of normal physiological activity, resulting in metabolic imbalances. These imbalances, together with the visual symptoms of chilling injury, are the means with which chilling injury is most frequently quantified and characterized (Walker et al., 1991).

One of the most sensitive responses of plants to chilling temperatures is the inhibition of photosynthesis (Allen and Ort, 2001). This inhibition can take several forms, from damage to photosystems II and I when leaves are illuminated with normal irradiances at moderately chilling temperatures (Kingston-Smith et al., 1997), the inhibition of CO_2 fixation following moderate chilling in darkness**,** to the loss of photosystem II function in darkness under more severe or prolonged chilling treatments (Allen and Ort, 2001; Kingston-Smith et al., 1997). Chilling-induced damage to photosystem can be readily determined by using the non-destructive measurement of chlorophyll fluorescence (CF).

In contemporary research, the most commonly encountered parameter derived from fluorescence measurements which is used to measure stress responses is the F_v/F_m ratio. The F_v parameter is equal to the maximum fluorescence yield (F_m) minus the minimal fluorescence yield (F_0), where Fm is the fluorescence yield when electron acceptors of PSII are reduced to the maximum level and F_0 is the fluorescence yield when electron acceptors of PSII are fully oxidized. The F_v/F_m ratio of unstressed dark-adapted plants is typically around 0.8 (Björkman and Demmig, 1987; Bolhàr-Nordenkampf et al., 1989) and its value is proportional to the quantum efficiency of photosystem II electron transport (Maxwell and Johnson, 2000). Chlorophyll fluorescence has been tested as a tool with which to assess chilling injury development in green plant tissues; it has many advantages in this regard as it is rapid, sensitive, non-destructive and it appears able to detect damage before any visible symptoms appear (DeEll, 1996; DeEll and Toivonen, 2003). Examples of fruits for which chlorophyll fluorescence has been

used successfully to identify chilling injury are banana (*Musa acuminata* Colla) and mango (*Mangifera indica* L.) (Smillie et al., 1987), cucumber (van Kooten et al., 1992), green pepper (*Capsicum annuum* L.) (Lurie et al., 1994) and in lemon fruits peel (Nedbal et al., 2000). Decreases in the F_v/F_m ratio can be used to measure the effects of chilling in the dark (Tijskens et al., 1994) and the light (Havaux and Lannoye, 1984). In older literature other parameters derived from fluorescence measurements are encountered, for example the decrease of F_R (F_i to F_p level of fluorescence) (Smillie and Hetherington, 1983; Smillie et al., 1987).

The loss of F_v/F_m following chilling in the light and in dark may well be due to different mechanisms. Under illumination, the loss of F_v/F_m is referred to as photoinhibition (strictly speaking the photoinhibition of photosystem II), and has been very extensively studied. It is due to photodynamic damage to the photosystem II reaction centre, and though the exact mechanism of this damage is not known, it appears that active oxygen species formed in the reaction centre are involved (Taylor et al., 1994).

The loss of F_v/F_m that occurs in darkness has been poorly studied and its mechanism is not understood. However the absence of light excludes the possibility of a photodynamic process. It is possible that changes in the phase of the thylakoid lipids may be involved in the dark loss of F_v/F_m even at temperature above the temperature of the dominant phase transition of the polar lipids of the thylakoid membrane (Meir et al., 1995; Parkin et al., 1989). A working hypothesis for the reduction of F_v/F_m in darkness at chilling temperatures is that either thylakoid membrane oxidation increases (Hariyadi and Parkin, 1991) or that the membrane transforms to a gel state; either of these processes will result in membrane dysfunction, and these two processes may act in combination. The loss of F_v/F_m that ensues is due to a reduction of F_m, not an increase in F_0. How this arises is not well understood in the context of dark chilling, but damage to the oxygen-evolving complex could explain the phenomenon.

In this article we aim to assess the development of chilling injury in the two mango varieties that are the most popular in the Egyptian market: Zibdia and Hindi-Be Sennara. These mangoes were harvested in different maturity stages in order to assess to what extent maturity stage influences sensitivity to chilling injury. Also, we intend to verify the possibility of using CF measurements as a tool for detecting CI sensitivity even before visible CI symptoms are evident. To this end we will use a range of techniques to measure chilling injury in order to be able to compare chlorophyll fluorescence with other techniques. Some mango varieties are known to be more tolerant to low storage temperatures than others (Ibrahim and Khalif, 1999) and Hindi-Be Sennara has been classed as more chilling tolerant than Zibdia.

2. Materials and methods

2.1. Fruit materials

Fruits from two mango (*Mangifera indica* L.) varieties, Zibdia and Hindi Be-Sennara, were harvested on 6th August 2002 from trees more than 20 years old growing in sandy soil. The farm was located in Sharqia, East Egypt (30.35 N and 31.30 E). Zibdia and Hindi Be-Sennara are poly-embryonic varieties. Fruits at three different harvest maturity stages were harvested from the shaded side of the trees when the average field temperature was 39^{o}C. The maturity stages were classified as: immature (M1), half-mature (M2) and fully mature (M3). This classification was based on the morphological development of fruit shoulder: in M1 fruits the shoulder is below the stem end, in M2 fruits the shoulder is at the same level as the stem end, and in M3 fruits the shoulder is above the stem end (Majeed and Jeffery, 2002). The fruits were collected and washed with water at 10 -13^{o}C to reduce both fruit temperature and the microbial load on the fruit surface. The fruits were then transported in a refrigerated vehicle to Cairo airport at 10-13^{o}C. The fruits remained for 21 hours in Cairo airport before being shipped to the Netherlands [a journey of 5 hours]. Following the arrival of the fruits in the Netherlands it took 50 hours to transfer the fruits to their temperature controlled storage rooms. During this period the fruits were held either at ambient north European summer conditions or for short periods at 5° C (Figure 1). Temperatures were recorded during the transport process using a miniature data logger (Escort Junior, Escort Data Logging Systems Ltd, Auckland New Zealand) placed amongst the fruits.

2.2. Storage conditions

Upon arrival in Randwijk, the 360 fruits were divided into 5 batches for non-destructive measurements (chlorophyll fluorescence and chilling injury index); each one was composed of 72 Hindi and 72 Zibdia fruits (24 fruits of each maturity stage in three replicates. Destructive measurements such as ion leakage was measured on two mango varieties (2160 fruits for Zibdia and 2160 fruits for Hindi) which were divided into 5 batches; each one composed 432 fruits (Zibdia 216 and Hindi 216 fruits), with 72 fruits of each maturity stages in three groups as replicates. 9 fruits of each maturity stage were picked every 5 days intervals for measuring ion leakage. These batches were stored at 5 different temperatures: 2, 4, 7, 10, and 20^{o}C. Chlorophyll florescence was measured every 3 days up to 36 days, and the CI index and ion leakage were measured every 5 days up to 35 days.

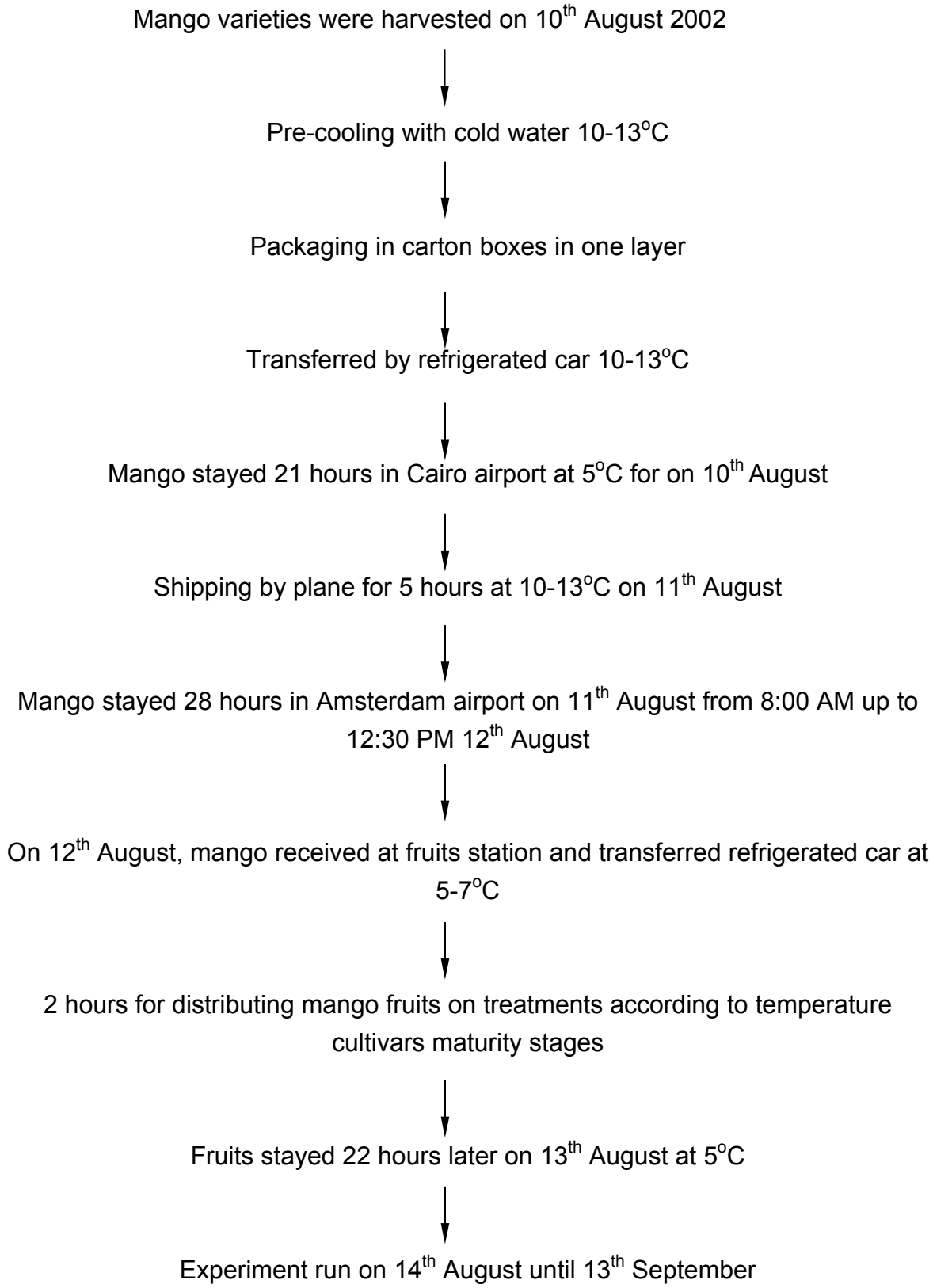

Figure 1.The logistic chains during three days of import two mango varieties to Netherlands 2002

2.3. Chilling injury index

A visual assessment of external damage, such as pitting, water soaked areas, and decay is often used to assess chilling injury. This approach is obviously subjective in nature, but frequently employed in routine assessments of product damage. In order to attempt to relate the more objective measurement techniques of chlorophyll measurements, electrolyte leakage and chlorophyll fluorescence to the visual assessment of injury we chose to score visual injury using the method of Chaplin et al (1991). This method requires that fruit from a batch are scored for injury according to the following scale: 0 = no damage (ND); 1 = very light damage (VLD); 2 = light damage (LD) (< 5% area affected); 3 = moderate damage (MD) (6-25% surface affected); 4 = severe damage (SD) (26-50% surface affected) and 5 = very severe damage (VSD) (>50% surface affected). The CI index is then calculated using the following formula:

CI index = $(1*FN_{VLD}/FN) + (2*FN_{LD}/FN) + (3*FN_{MD}/FN) + (4*FN_{SD}/FN) + (5*FN_{VSD}/FN)$

Where VLD, LD, MD, SD and VSD were the percentage of fruits presenting the different degrees of CI and the FN means the total fruits in treatment. The CI index was measured every 5 days up to a maximum of 35 days.

2.4. Chlorophyll fluorescence

CF data were recorded from individual dark-adapted fruits. Measurements were collected from four positions per fruit and were made every three days using a commercial fluorimeter (Mini-PAM, Walz, Effeltrich, Germany) controlled using data acquisition software supplied with the fluorimeter (Win Control, Walz, Effeltrich Germany). The parameters recorded were F_0 (minimal fluorescence), F_m (the light-saturated yield of fluorescence) and the F_v/F_m ratio (the ratio of variable fluorescence to maximum fluorescence, where $F_v = F_m - F_0$). Damage can be inferred by a decrease in the F_v/F_m ratio below 0.75 - 0.78, which in biophysical terms implies a reduction in the photochemical conversion efficiency of photosystem II (Toivonen and DeEll, 2001).

2.5. Chlorophyll content

Samples of 12 fruits were collected at 5-day intervals and the samples were divided into peel and pulp. Samples were stored at –80^{o}C. To avoid photo-oxidation of chlorophylls all preparations were carried out in the dark. The peel samples were frozen at -80^{o}C and lyophilized under a strong vacuum for 7 days. The lyophilized samples stored in desiccators at room temperature in the dark. The extraction chlorophyll method of Mónica et al. (1994) was modified by using N,N-

dimethylformamide (DMF) instead of acetone. The samples were then ground to powder in a ball mill. To 0.8 g of ground powder, 5 ml (DMF) was added. The suspension was sonicated for 15 min at 4°C and then stored at 4°C for 16 hours to allow the DMF to leach the pigments from the sample (Minguez-Mosquera et al., 1991). Finally, 1 ml of the supernatant was centrifuged for 5 min at 16000 rpm and 4°C to remove any suspended material and the clarified supernatant was then analyzed by HPLC. This system consisted of a P580 pump (Dionex,), a MIDAS auto-sampler (Spark, Emmen, The Netherlands) and a UVD 340S diode array detector (Dionex, Sunnyvale, USA), all linked to Chromeleon chromatography software (Dionex, Sunnyvale, USA). Separations were carried out at 30°C using a 250mm long 4mm diameter LiChrospher 100 RP-18 (5 μm) column (Merck, Darmstadt, Germany). Chlorophyll was separated with gradient elution at a flow of 1.2 ml min^{-1}, using acetonitrile: methanol: water (84:9:7 v/v) (solvent A) and methanol: ethyl acetate (68:32 v/v) (solvent B). The gradient was as follows: 0-12 min, 0-100% B; 12-18 min, 100% B (García-Plazaola and Becerril, 1999). Chlorophyll was detected at 445 nm.

2.5. Ion leakage

Samples of 9 fruits were collected at 5-day intervals. Disks (7 mm diameter) of peel and pulp tissue were cut from five different parts of each fruit using a cork-borer. The disks were washed three times in demineralized water and placed in 10 ml 0.4 M mannitol in demineralised water at 24°C for 3h (Hakim et al., 1999). Electrical conductivity of the aqueous phase was measured using a conductivity meter, after which the tissue samples were killed by heating in water bath at 100°C for 20 minutes. This cooking process allows the release of all electrolytes from the tissue. Once cooled to room temperature the conductivity was re-measured and the relative electrolyte leakage from the uncooked pulp and peel samples was calculated as follows:

$$\text{IL}\,(\%) = \frac{\text{Conductivity}_{\text{after 3 h}}}{\text{Conductivity}_{\text{after boiling}}} \text{ x } 100$$

2.6. Statistical analysis

Data for the evolution of CF parameters in term (F_m, F_0 and F_v/F_m ratio), CI-index, chlorophyll and ion leakage in time were analyzed using analysis of variance (ANOVA), with three factors for temperature, varieties, and harvest maturity stage. Means were compared using the least significantly differences (L.S.D.) at p=0.05. Data were analyzed for two periods: the first is from 0-20 days, when all treatments were present and the second is from day 0-35 days for all treatments except for

storage at 20°C, for distractive measurement. Linear regression analysis and ANOVA were analyzed at the 5% probability level. The statistical software package Genstat 8 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) was used.

3. Results

3.1. Chilling injury index

The chilling injury index, as a function of storage time in days for all the maturity stages of both varieties of mango at different temperatures is shown in figure 2. CI index shows a significant interaction at $p \leq 0.05$ when the storage time, temperature, varieties and maturity stages were considered. Considering the different maturity stages, it is clear that the immature stage (M1) of both varieties is more resistant to damage compared to the other maturity stages.

At storage temperatures of 2°C and 4°C there was no evidence for CI before 10 days of storage in all the maturity stages and for both the varieties used in this study. In the case of the fruit stored at 7°C no injury was detected before 20 days. Storage at 10°C produced no signs of injury within the 35 days of the experiment irrespective of the cultivar or the maturity stage. The first symptoms that appeared after 10 days or 20 days were black spots on the peel of the fruit. Though an increase in storage temperature from 4°C to 7°C increased the time delay before development of injury, the degree of damage that developed between 10 and 20 days was slight. As a result of this minor development of injury before 20 days there was only a weak effect of storage temperature on damage development before 20 days irrespective of the variety or developmental stage.

After 20 days differences emerged between the cultivars and maturity stages with respect to the temperature dependency of damage. After 20 days of storage CI developed more rapidly in Zibdia than in Hindi be-Sennara. With increasing duration of storage it also became clear that chilling at 2°C and 4°C produced the greatest degree of chilling injury, with the 4°C treatment being more damaging than the 2°C treatment. At 7°C the degree of injury was in general less severe and no symptoms injury developed at 10°C and 20°C. The sensitivity of Hindi to injury was independent of maturity stage, whereas the sensitivity of Zibdia increased as the fruits became more mature.

3.2. Chlorophyll fluorescence parameters

In figure 3, the F_v/F_m ratio of both mango fruits was plotted as a function of storage time at different storage temperatures. A significant interaction is found when temperature and varieties are considered.

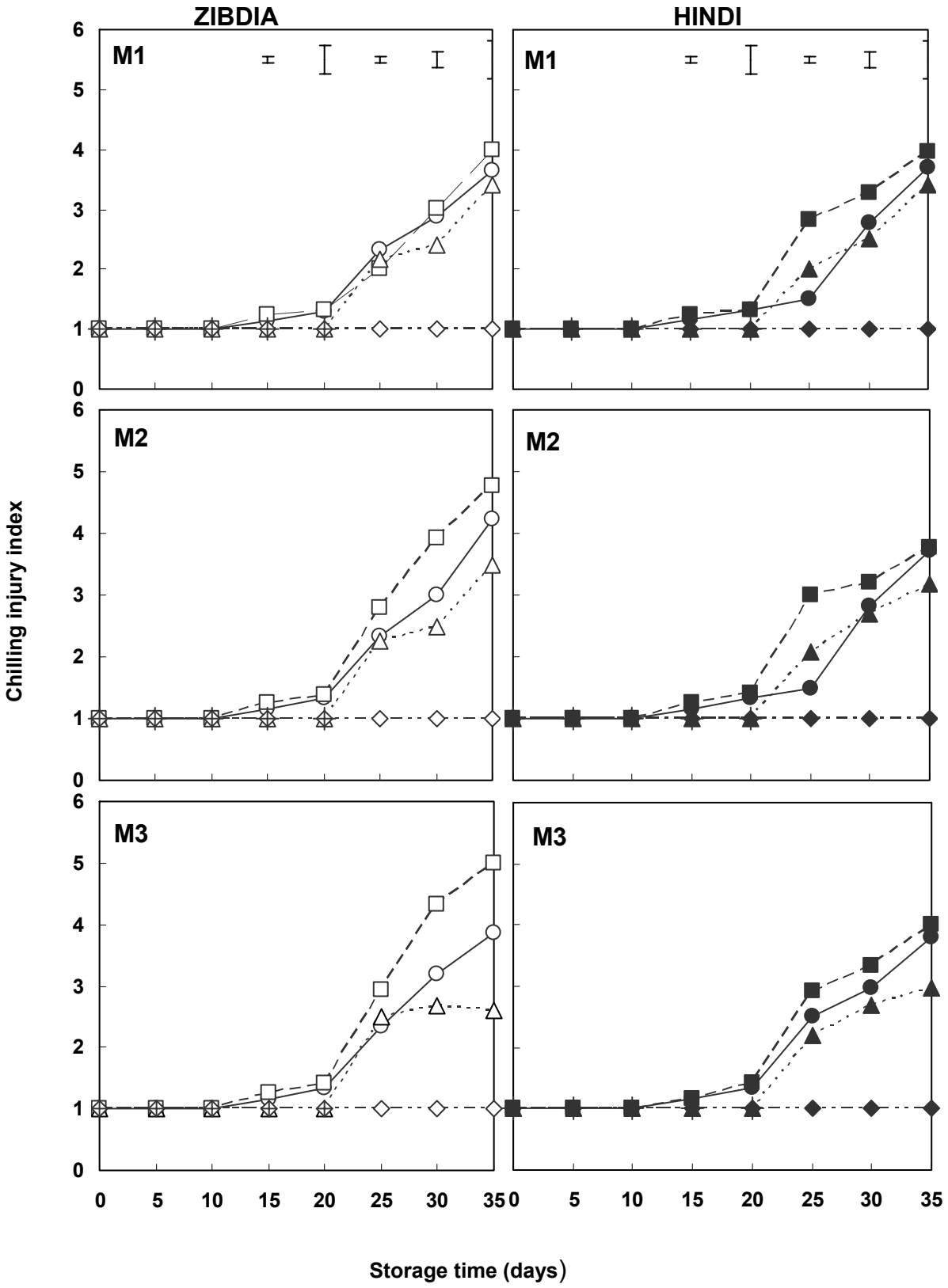

Figure 2. Chilling injury index versus storage time of two mango cultivars Zibdia and Hindi harvested at different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3) as a function of storage time at different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■- 4, -▲- 7, -◆- 10 and -+- 20^{0}C). Chilling injury index expressed as mean (n=3). The Vertical bars indicated to L.S.D. (p=0.05).

It is clear that overall there is a significantly greater decrease in the Fv/F_m Zibdia compared to Hindi. With the exception of the 20°C treatment, the decrease of Fv/F_m in Hindi appears linear and gradual, with a trend to a greater loss of F_v/F_m at lower temperatures, though the 4°C treatment always had a lower F_v/F_m than the 2°C treatment (not statistically significant). The F_v/F_m from the 20°C treatment followed the same trend as all the other treatments until around day 9 after which it decreased more rapidly to about 0.45 at days 18 and 21. After day 21 the measurements on the fruits stored at 20°C ceased owing to their deterioration.

Compared to Hindi, Zibdia showed a different pattern of change of F_v/F_m against storage time. It is clear that for storage temperatures of 2°C, 4°C and 7°C F_v/F_m initially decreased in a manner similar to that shown by Hindi, but after a certain time F_v/F_m began to decrease much more rapidly. This break in the F_v/F_m temperature relationship occurred at about 21 days for the fruits stored at 2°C and at about 30 days for the 7°C fruits. In the case of the 4°C fruits the point of the break is less easy to determine, but it could be as low as 6 days. It is clear that the 4°C storage temperature produces a greater decrease in F_v/F_m than 2°C, though at no single temperature is this difference significant. The 20°C storage temperature produced in Zibdia, as it did in Hindi, a rapid loss of F_v/F_m though in this case the decrease was comparable to that shown by the 4°C treatment. Notably, though at 20°C for a decrease of Fv/Fm to around 0.55 in Zibdia marked the end of the storage life of the fruit, during chilling a much lower Fv/Fm could be achieved.

Both F_M and F_0 values increased in both varieties in the initial storage period up to day 12 (Figure 4), and in the case of both F_M and F_0 this increase was significant. Regarding the effect of the storage temperature on F_0 values, it was found that the values increase as the storage temperature decrease regardless of the type of mango. F_0 values change according to the storage period. In the first 10 days and at all the storage temperatures, the values are almost doubled. During the storage period 12-24 days, F_0 decreased gradually almost to the original values, after which there was no further significant change irrespective of the storage temperature. Hindi fruits stored at 20^{o}C presented the highest F_0 values compared with fruits stored at temperatures from 2 to 10^{o}C, nevertheless, Zibdia fruits showed higher increase in F_0 in comparison to the fruits stored at 7 and 10^{o}C.

F_m increased significantly ($p<0.05$) after day 6 and up to day 12 independent to storage temperatures, followed by a steady decrease until the end of the storage time (36 days) and the decrease was more marked in Zibdia compared to Hindi. After the 12 day maximum, larger decrease was observed in Zibdia fruits stored at

2 and 4°c compared to fruits stored at 7 and 10°C. Fruits stored at 20°C showed decreases in Fm beginning on day 9 and extending to day 21 (Figure 5).

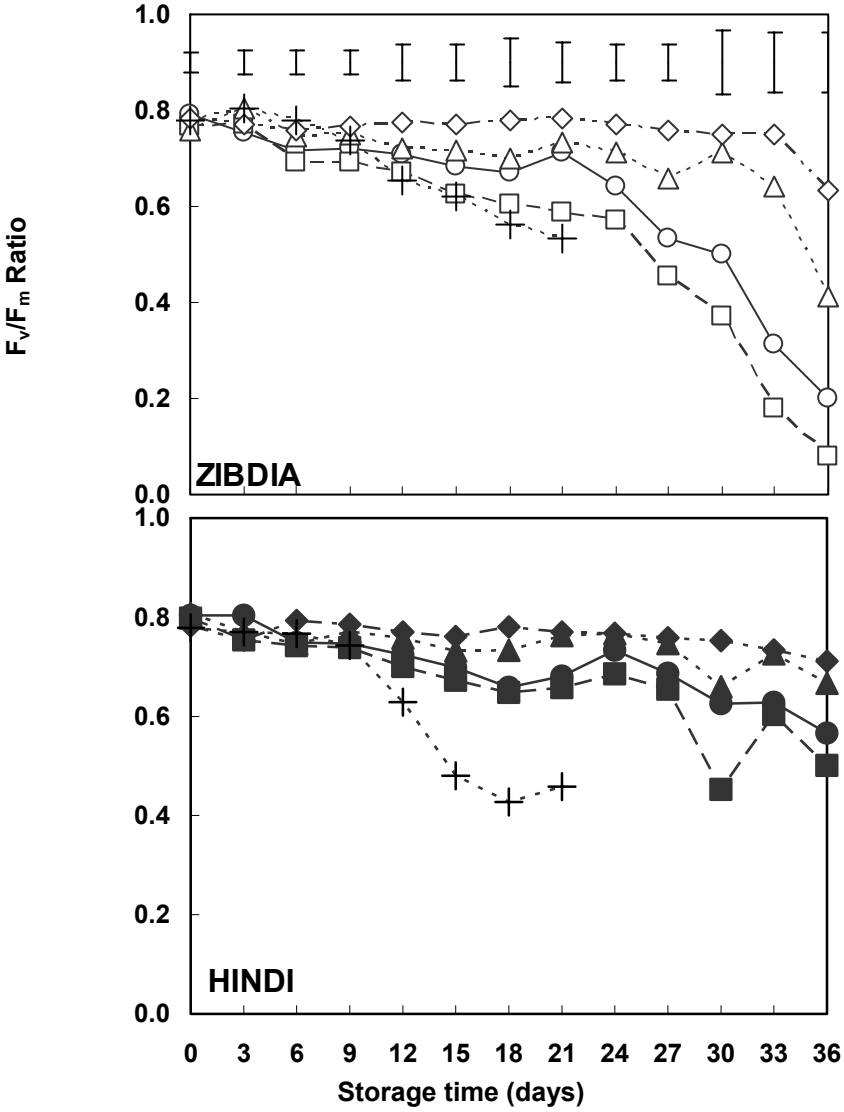

Figure 3. F_v/F_m ratio of Zibdia and Hindi of mango cultivars as a function of storage time at different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). F_v/F_m ratio expressed as mean (n=3). The vertical bars indicated to L.S.D. at *p*=0.05.

3.3. Total Chlorophyll AB content and A:B ratio

Figure 5 depicts the variation of total chlorophyll (Chl_{ab}) in both varieties as a function of storage time, at different storage temperatures. The interaction ($p<0.001$) was significant among days, temperatures, maturity stages and varieties. Chl_{ab} content decreased with storage duration at the different maturity stages for both varieties. In comparison to Zibdia, Hindi fruits initially contained less Chl_{ab} at all the maturity stages (~30 *vs* ~55 mg/100 g^{-1} DW).

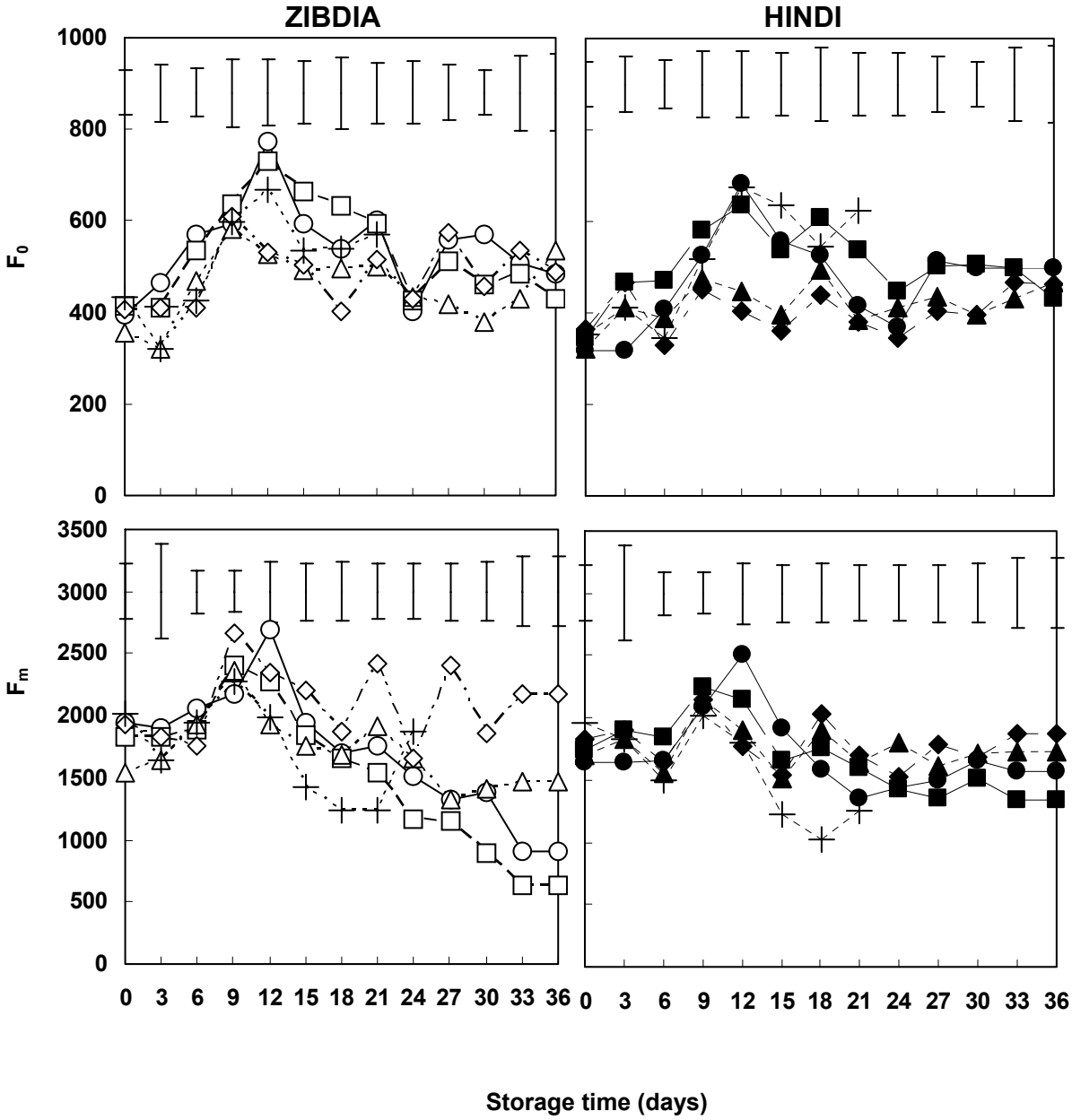

Figure 4. Presents the F_0 and F_m parameters of two mango varieties Zibdia and Hindi as function of storage time at different storage temperatures. Symbols represent the storage temperatures are (Zibdia:-○-, -□-4, -△- 7, -◇- 10 and - +- 20°C) and (Hindi: -●- 2, -■- 4, -▲- 7, -◆- 10 and -+- 20°C). F_0 and F_m expressed as mean (n=3). The verticals bars indicated to L.S.D. at *p=0.05*

However, the final Chl_{ab} content was largely the same in both varieties, with the exception of the 10°C Zibdia fruits, which retained considerably more chlorophyll than the other Zibdia fruits. The effect of storage temperature on theChl_{ab} content of Hindi was not significant, except for the 20°C control which lost chlorophyll more rapidly than the cold stored fruits. In the case of Zibdia there was a noticeable effect of different low storage temperatures. As noted above, the 10°C Zibdia retained the most chlorophyll irrespective of the initial maturity stage of the

fruits, the 2°C and 7°C fruits had similar amounts of Chl_{ab}, and the least chlorophyll was present in the fruits stored at 4°C.

For both Zibdia and Hindi fruits stored at 20°C and irrespective of the maturity stage, Chl_{ab} decreased rapidly during storage, faster than in any other temperature. Other than the higher initial Chl_{ab} content, there did not appear to be a marked effect of maturity stage on the response of Chl_{ab} content to storage.

Figure 6 shows the chlorophyll a/b ratio ($Chl_{a/b}$) plotted against the storage time for both varieties of mango. The initial $Chl_{a/b}$ initial values are the same for both varieties at all the maturity stages. However during storage there are interesting differences between the two varieties which are independent of maturity stage at time of harvest. For Zibdia the $Chl_{a/b}$ ratio was in nearly all cases (M1 fruits stored at 4°C and measured at 35 days was the only clear exception) largely unchanged during the storage. Hindi, on the other hand, showed increases in the $Chl_{a/b}$ ratio after about 15 days of low temperature storage and 10 days at 20°C.

3.4. Ion leakage

Figure 7 shows the ion leakage percentage plotted as a function of storage time (days) at different storage temperatures for both the peel and the pulp of Zibdia and Hindi mango fruits at three different maturity ages. It is apparent from the figure that fruit peel has less ion leakage compared with fruit pulp. In very broad terms it is clear that ion leakage increases with storage duration, but when considered in more detail there are some interesting differences between cultivars, maturity stages, and treatments. As with the other measurements described above, the responses of the fruits stored at 20°C were generally different to those of fruits stored at reduced temperatures. With the exception of the Zibdia M3 peel and the Hindi M1 peel, leakage increased more rapidly from the 20°C fruits than any other treatment. After 20 days of storage the condition of these fruits became so bad that no further measurements could be made upon them. For fruits stored at low temperatures ion leakage had an overall linear or curvilinear increase with time. Leakage from the peel of either cultivar was not strongly affected by temperature in maturity stages M1 and M2, but in the M3 stage there was increased temperature dependency in both cultivars. In the M3 stage it was clear that for both Zibdia and Hindi 4°C storage produced the greatest increase in leakage at low storage temperatures, followed by storage at 2°C. In neither cultivar was the initial leakage from the pulp tissue dependent on maturity stage. For both cultivars and all maturity stages the least increase leakage of the pulp tissue at the end of the measurement period was shown by fruits stored at 10°C. The greatest leakage was in most cases provoked by storage at 4°C, or more rarely 2°C. Where the greatest damage was caused by 4°C storage, the next most severe damage was caused by storage at

2°C. Maturity stage at harvest had no clear effect on the progression of the increase in leakage during storage.

3. Discussion

The chlorophyll fluorescence based F_v/F_m parameter has, along with other parameters derived from fluorescence or other biophysical probes of photosynthesis, a long history as a tool for assessing stress and damage in green plant tissues. The results obtained in this study show that in Zibdia, and to a lesser extent in Hindi, cold storage produces changes in F_v/F_m that are temperature dependent (Figure 3). Thus according to changes in F_v/F_m parameter Zibdia is more chilling sensitivity than Hindi. Thus, this simple fluorescence parameter is able to detect temperature induced damage that develops in darkness in photosystem II. The changes in F_v/F_m cannot be substantially attributed to an increase in F_0; though F_0 increases with time of storage, so does F_m, and the increase in F_0 is insufficient to account for the large losses of F_v/F_m.

Changes in the absolute values of fluorescence yield are difficult to interpret whenever these changes occur simultaneously with changes in chlorophyll content. However, figures 3 and 4 do give some insights into the underlying causes of changes in F_v/F_m in terms of F_0 and F_m (Figure 8 and 9). Under low temperature conditions, the decreases in F_v/F_m of Hindi fruits are correlated with small increases in F_0 and larger increases in F_m. Such a small increase in F_0 is frequently observed during the decreases of F_v/F_m that occur in the early stages of light-induced photoinhibition experiments on photosystem II. At 20°C, Hindi fruits behave in a similar way as they do under cold-storage, except that the changes in F_0 and Fm are more extreme (Figure 4). Zibdia fruits at 20°C behave in a similar way to Hindi fruits, but under cold storage their behaviour is more complex. The decreases in F_v/F_m that occur in Zibdia fruits are larger than encountered with Hindi fruits, and these large decreases are closely correlated with decreases in F_m (Figure 4), which is consistent with the results obtained from Hindi. The relationship between F_0 and F_v/F_m from Zibdia is more complex than that found with Hindi; at 10°C and 7°C the responses are similar to Hindi, but at 4°C and 2°C some samples show a response similar to Hindi whereas others show large decreases of F_v/F_m that are independent of changes in F_0, so overall there is no correlation between F_0 and F_v/F_m. Based on the visual assessment of chilling injury (the CI index) the following conclusions can be drawn:

- Initially (M1 stage) Hindi and Zibdia have an equal degree of chilling tolerance
- With increasing maturity Zibdia becomes more chilling sensitive than Hindi

- In all cases the greatest degree of chilling injury developed at 4°C and not 2°C.
- Storage at 10°C produced no signs of injury

Increases in the degree of sensitivity to the development of visual symptoms of injury can occur as mango fruits mature (Majeed and Jeffery, 2002), but the extent to which this happens varies from cultivar to cultivar. This would appear to be an important physiological property from the point of view of both breeding and selecting the best cultivars when storage is required. Also, at least as far as deterioration of the visual appearance of the fruit is concerned, relatively prolonged storage at 10°C is possible, but below this temperature damage will develop with decreasing temperature (González-Aguilar et al., 2000; Nair et al., 2003). Based on the changes to the visible appearance of the fruits, the report that Zibdia is more chilling sensitive than Hindi (Ibrahim and Khalif, 1999), is only correct at later maturity stages; or put another way, if harvested early Zibdia is as chilling tolerant as Hindi. It is surprising that the lowest temperature did not produce the greatest degree of injury; this pattern was also observed in the responses of F_v/F_m, chlorophyll content (in Zibdia) and, to a lesser degree, electrolyte leakage so it is a general phenomenon. A simple interpretation of this response is that though low temperatures produce chilling injury, the development of that damage is temperature dependent (Majeed and Jeffery, 2002). In the case of mango fruit this could result in less damage developing at 2°C compared to 4°C.

According to the changes in F_V/F_M Zibdia is more chilling sensitivity than Hindi. The CI-index data suggest that Zibdia only in the M2 and M3 stagesis more chilling sensitivity than Hindi. Other measurements of chilling injury do not, on the whole, strongly support the proposition that Hindi is more chilling tolerant than Zibdia. Though Hindi looses its chlorophyll more slowly than Zibdia, Zibdia has much more chlorophyll to start with. Because of the relatively good retention of chlorophyll in the 10°C Zibdia fruits of all maturity stages, this cultivar has a relatively greater variation in its chlorophyll retention than does Hindi. It is not clear, however, that this means that Zibdia is more tolerant than Hindi. The degree of ion leakage from either the pulp or the peel also does not indicate any greater chilling tolerance in Hindi compared to Zibdia. These different results raise problem for the determination of chilling tolerance. Depending on the technique chosen, the results differ in detail. Some techniques, for example CI-index, reveal interesting variation in chilling tolerance that could be of value in breeding

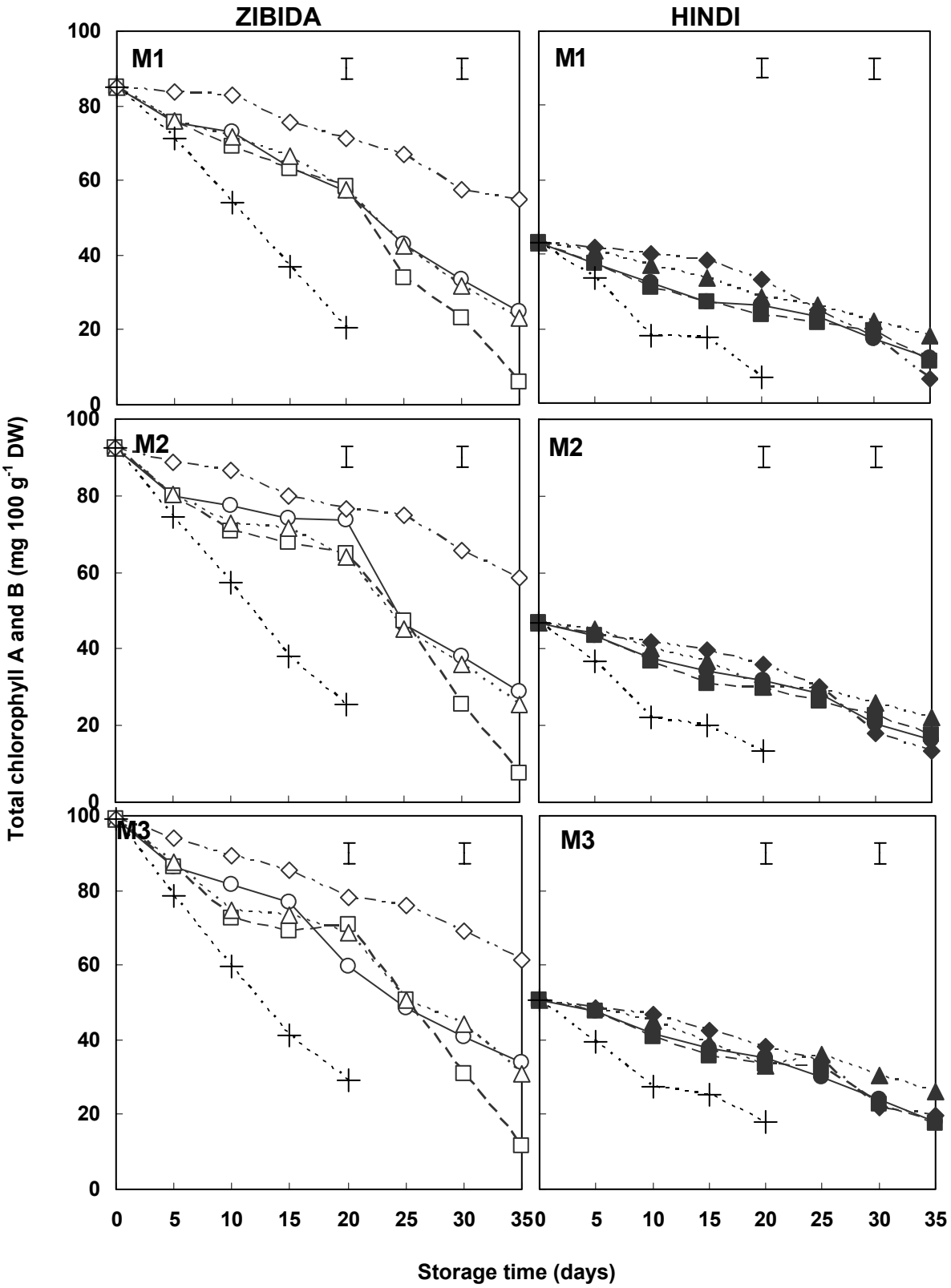

Figure 5. Total chlorophyll A and B (Chl_{ab}) content of Zibdia and Hindi mango cultivars which were harvested at different maturity stages: immature (M1), half-mature (M2), and fully mature fruits (M3) versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, - ▲- 7, -◆ 10 and -+-20^{0}C). Chlb content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

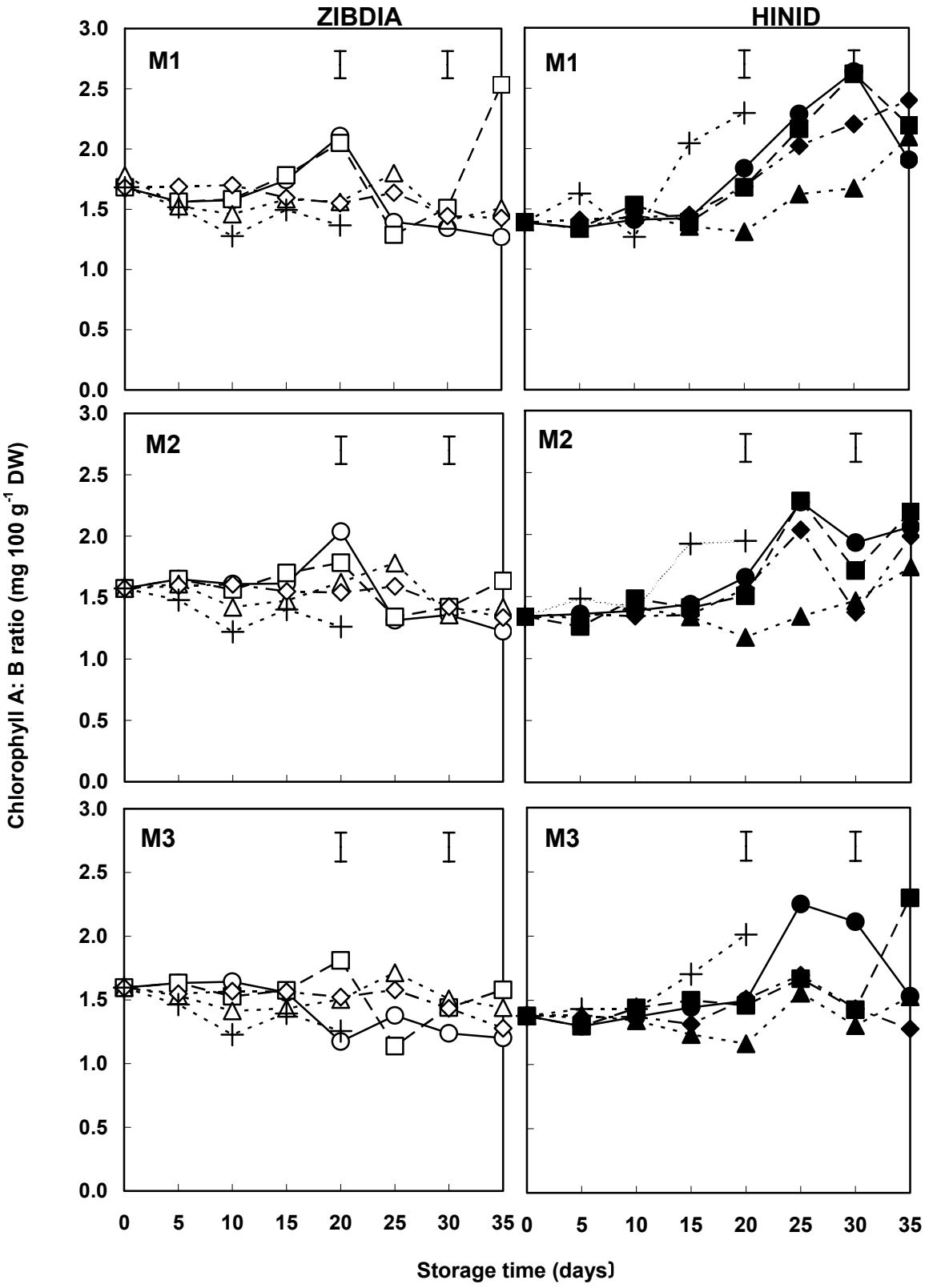

Figure 6. Chlorophyll A:B ratio of Zibdia and Hindi mango cultivars harvested at different maturity stages: immature (M1), half-mature (M2) and fully mature fruits (M3) versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). The ratio expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

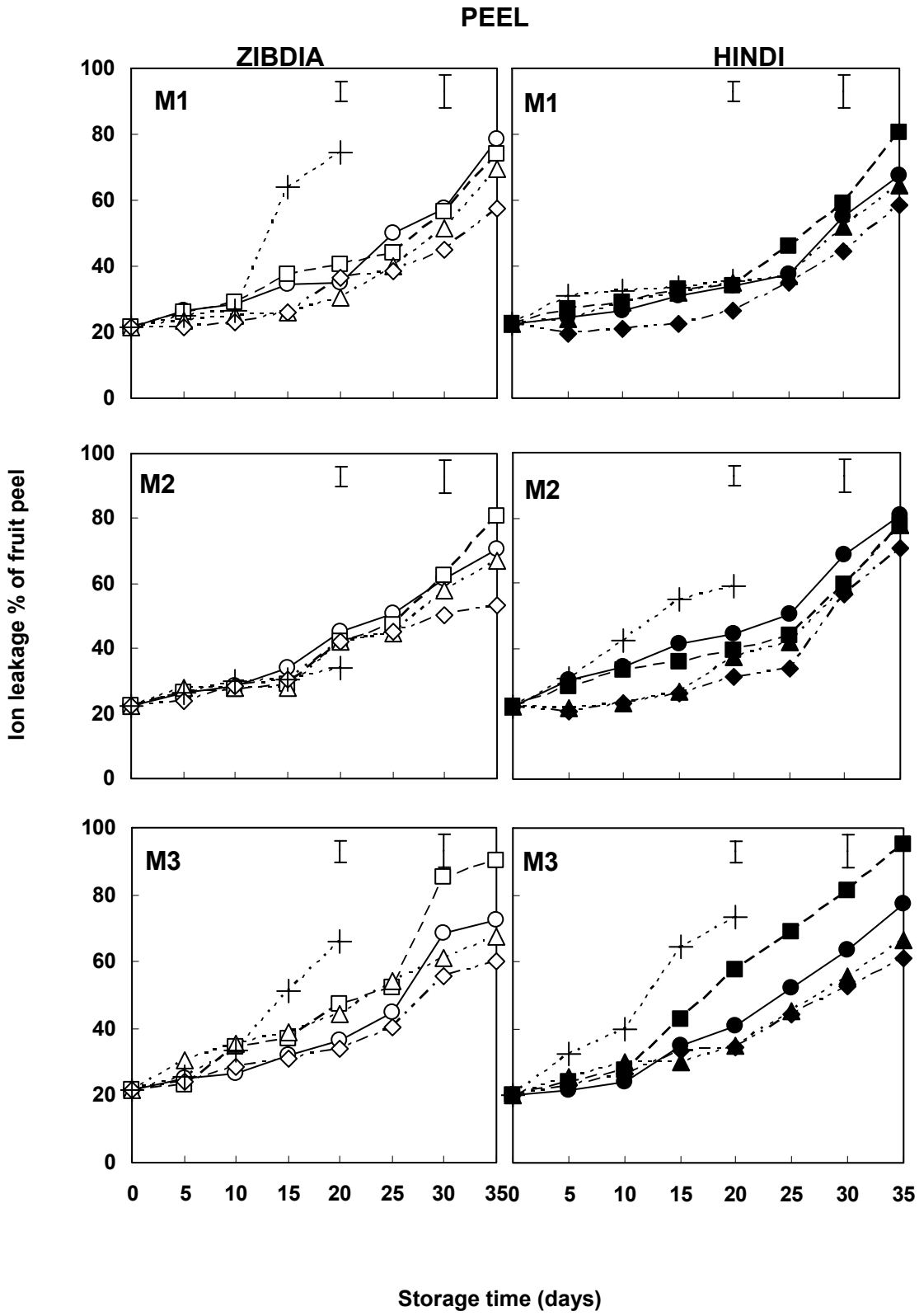

Figure 7a. Ion leakage percentage of fruits peel versus storage time of two mango varieties Zibdia and Hindi harvested at different maturity stages: immature (M1), half-mature (M2), and full mature fruits (M3) as a function of storage time at different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, -■- 4, -▲- 7, -◆- 10 and -+-20^{0}C). CO_2 expressed as mean (n=3). The Vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

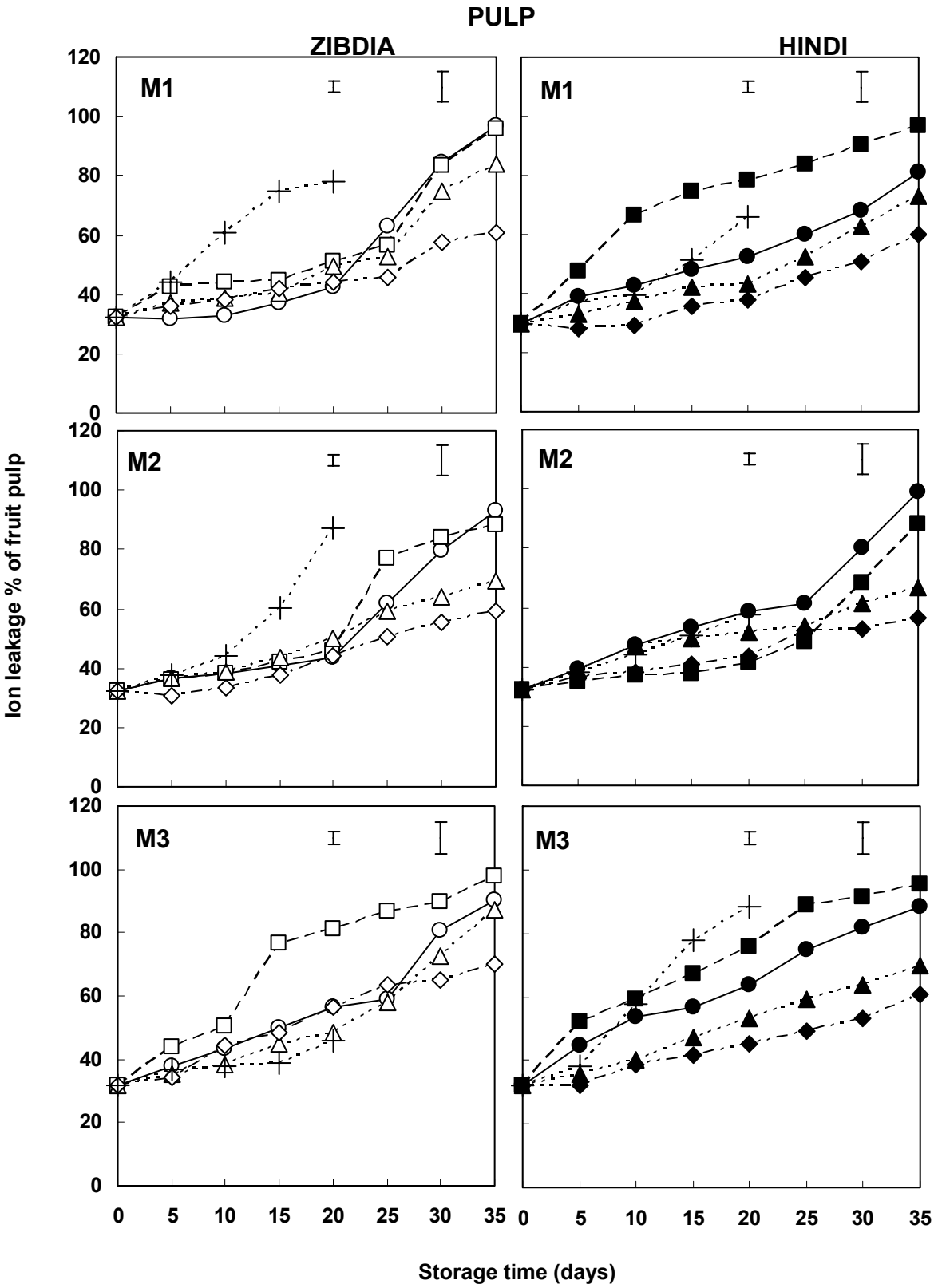

Figure 7b. Ion leakage percentage of fruits pulp versus storage time of two mango varieties Zibdia and Hindi harvested at different maturity stages: immature (M1), half-mature (M2), and full mature fruits (M3) as a function of storage time at different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, -■- 4, -▲- 7, -◆- 10 and -+-20^{0}C). CO_2 expressed as mean (n=3). The Vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

programs: in Hindi chilling tolerance is independent of maturity stages, whereas for Zibdia it decreases with increasing maturity. This pattern is not clearly revealed by other assays. This implies that case needs be taken when comprising chilling tolerances that have been evaluated by different techniques. It may be, however, that a more detailed analysis of the results obtained with the different assays will reveal patterns that are not evident in the time dependencies of the assays.
In qualitative terms the measures of chilling injury support each other, a quantitative comparison reveals a more complex relationship. A comparison of the CI-index with F_v/F_m (Figure 10) shows that there are three classes of relationship.

Fruits stored at 10°C show a vertical distribution of points, which reflects the small decrease of F_v/F_m at 10°C coupled with the lack of change in CI-index. Under these conditions the F_v/F_m measurement reveals damage to PSII that is not paralleled by changes in the CI-index. The second type of response is found at all other temperatures for Hindi and at 7°C for Zibdia. Here there is a large increase of the CI-index data which is correlated with only a small change in F_v/F_m, so F_v/F_m is nearly independent of CI-index. The third class of response is found only at 2°C and 4°C for Zibdia. Here there is a good correlation between CI-index and F_v/F_m. These correlations, or lack of them, imply that if CI-index is taken to be a good yardstick for measuring CI then F_v/F_m is a poor tool for measuring injury except in certain limited cases.
This pattern of results is also found in the relationship between F_v/F_m and both total chlorophyll ab content and electrolyte leakage (Figures 11 and 12). In this F_v/F_m only correlates well with ion leakage and chlorophyll content in Zibdia at 2°C and 4°C. The correlations obtained between F_v/F_m and electrolyte leakage for the control, un-chilled fruits of Zibdia and Hindi are, however, good, and for chlorophyll a content and F_v/F_m, reasonably good. So, on the one hand it is clear that changes in F_v/F_m are not, in general, a good indicator of chilling injury in dark-stored mango fruits except under relatively severe treatments of a susceptible cultivar. On the other hand, however, it seems that changes in F_v/F_m could be used just as well as changes in either chlorophyll content or electrolyte leakage to monitor the normal ripening process that occurred at 20°C (Jacobi et al., 1998). In conclusion, the generally poor correlation that exists between F_v/F_m and other measures of change during chilling conflicts strongly with other results that support the use of F_v/F_m as a measure of chilling injury (Meir et al., 1995). In contrast to the generally poor correlation that was found between F_v/F_m and CI-index, the relationship that exists between CI-index and electrolyte leakage from either the pulp or peel is good (Figures 10 and 12). Likewise, the relationship between CI-index and total chlorophyll content (Figure 13) is very close, though it differs between varieties

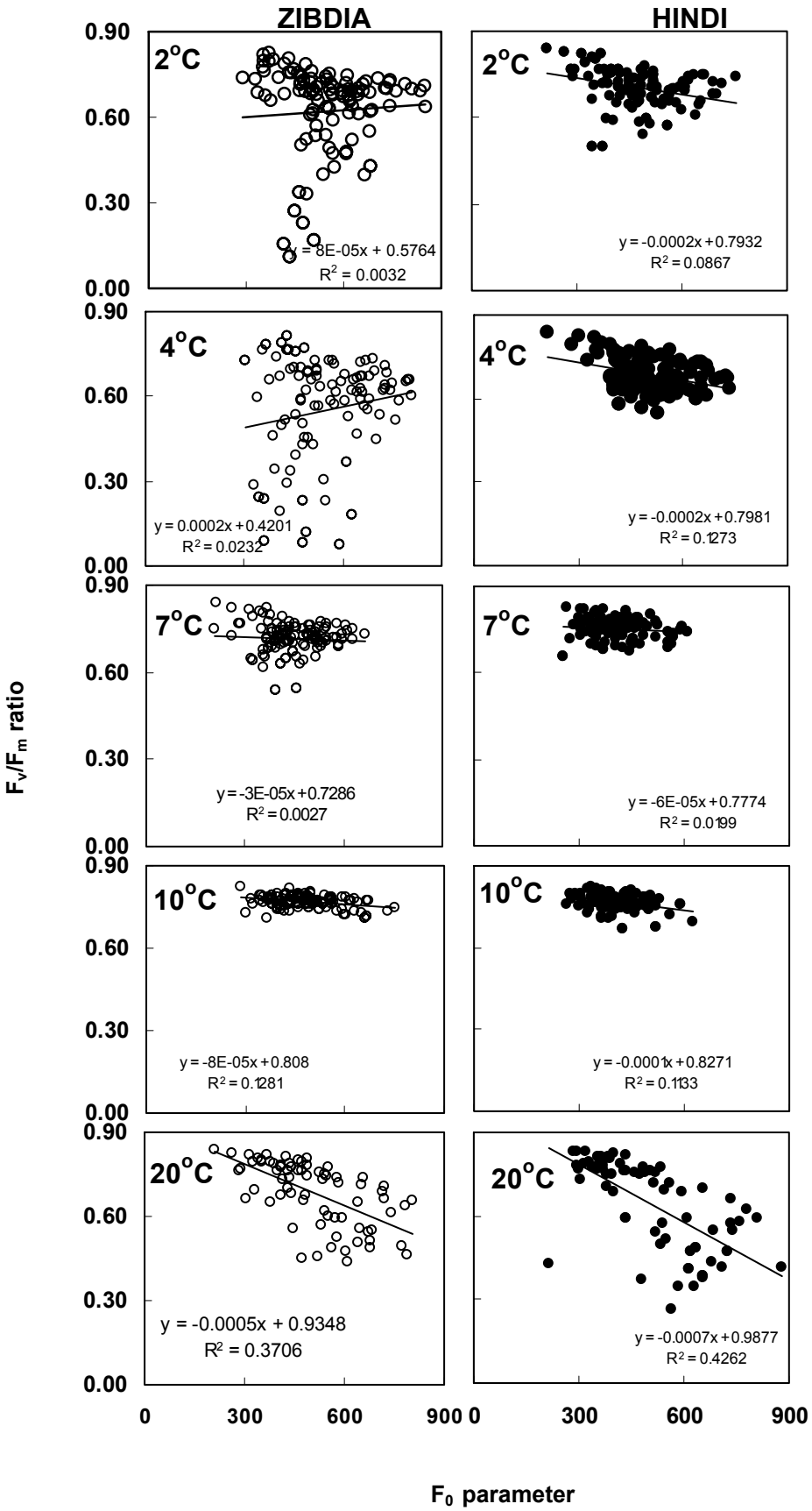

Figure 8. The correlation F_v/F_m ratio parameter in function of F_0 of tow mango cultivars 'Zibdia' and 'Hindi' which stored at different storage temperatures (2, 4, 7, 10 and 20°C) for 36 days. The lines represent linear regressions on the data.

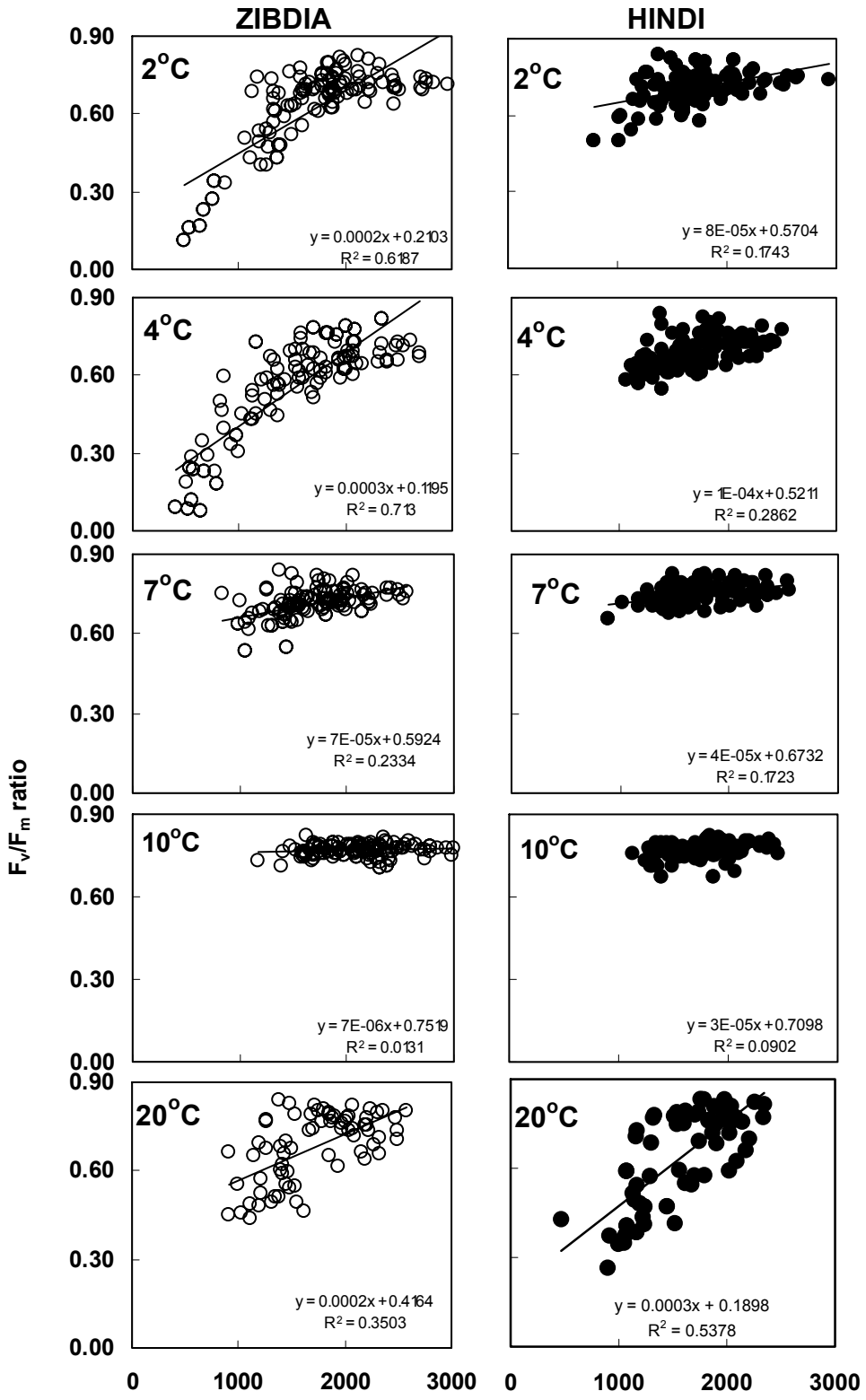

Figure 9. Present the correlation F_v/F_m ratio parameter in function of F_m of tow mango cultivars 'Zibdia' and 'Hindi' which stored at different storage temperatures (2, 4, 7, 10 and 20°C) for 36 days. The lines represent linear regressions on the data.

owing to the greater size of the changes in chlorophyll content that occur in Zibdia compared to Hindi. This problem of processes of change occurring over different ranges of values has been addressed by the batch-analysis approach of Schouten et al (2002). It seems reasonable to propose that this method could be adapted to eliminate the problem of differing initial chlorophyll contents complicating the use of a chlorophyll content measurement to assess chilling damage development during mango storage. Comparing different developmental stages from both Zibdia and Hindi subjected to different chilling treatments, there is always a similar, consistent positive correlation between CI-index and ion leakage from either the pulp or peel. It seems therefore that CI-index, electrolyte leakage, and changes in chlorophyll content are a homogeneous group of measurements for chilling injury. Overall the correlations are unaffected by maturity stage or temperature. The match is not, however, perfect; for example in Zibdia chilled at 4°C there is an influence of maturity stage on the relationship between leakage from the peel and CI-index (Figure 14a), just as there is at 4°C and 7°C for leakage from the pulp of Zibdia and CI-index (Figure 13b).

The F_v/F_m parameter responds quantitatively differently to the members of this group, though in qualitative terms it does still offer a means of identifying chilling damage. Of course all of these measurements are physiological in nature and do not say anything about product flavour etc. However in spite of this last reservation, it might be worth exploring tools for chilling injury assessment based upon colorimetric analysis, image analysis, or bio-impedance measurements as this measures are based upon process that are measured by the CI-index, chlorophyll content and ion leakage technique, or to explore more sophisticated applications of fluorescence.

The $Chl_{a/b}$ ratio responds differently to chilling in the two cultivars investigated. In the case of Zibdia the relatively constant ratio that is maintained during large reductions of chlorophyll content implies that the loss of chlorophyll is evenly distributed across all components, whereas with Hindi the increasing ratio suggests a preferential breakdown of Chl_b containing chlorophyll binding proteins, such as the LHC of photosystem I or II (DeEll and Toivonen, 2000; Rosenqvist and van Kooten, 2003).

Acknowledgement

The author would like to appreciate the Egyptian High Educational Ministry for support the PH.D., full program. Also Dr. Ep Heuvelink for statistical analysis advice.

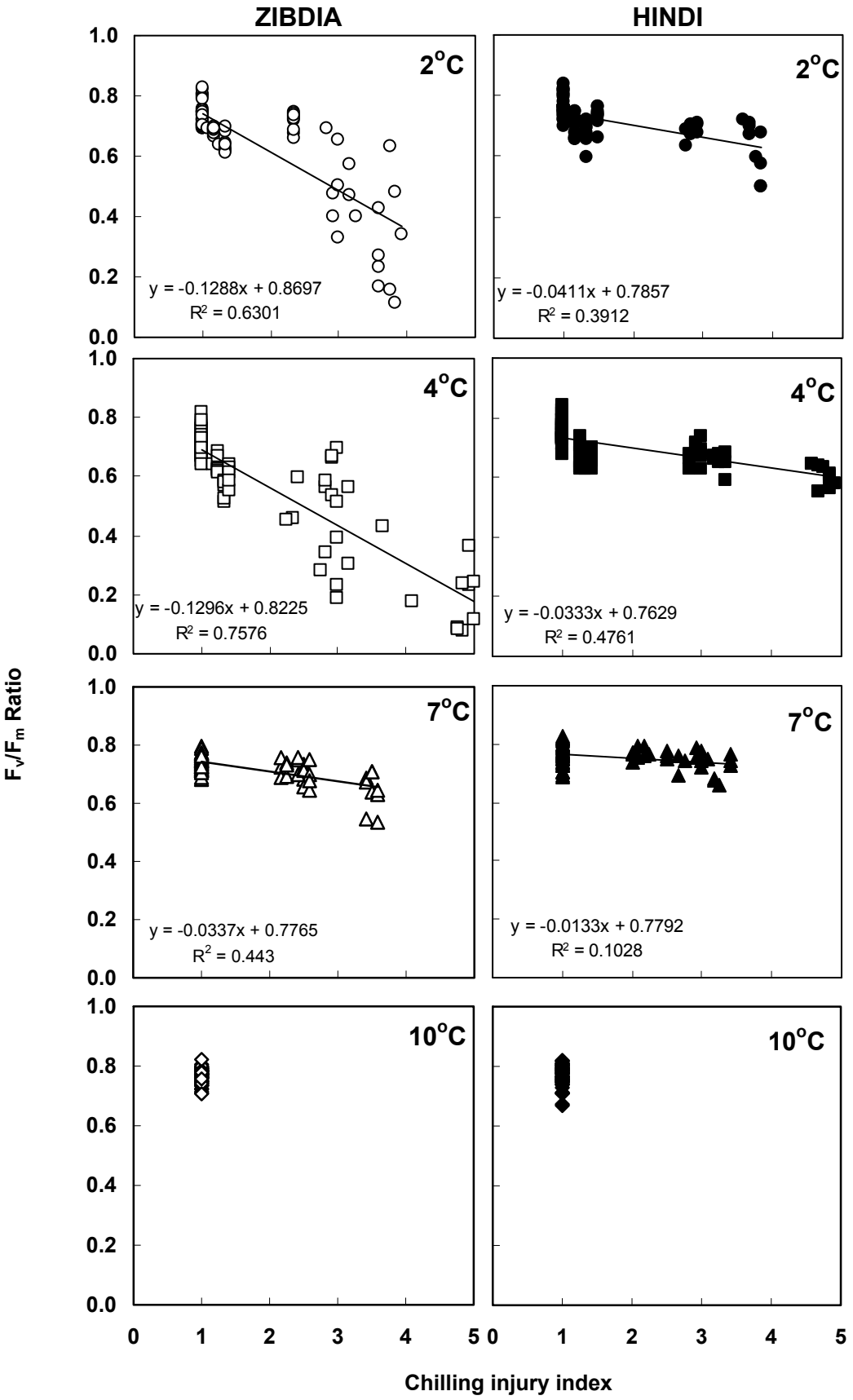

Figure 10. The correlation F_v/F_m ration in function of CI-index of Zibdia and Hindi at different storage temperatures for 35 days (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10^0C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10^0C). Solid lines represent linear regressions.

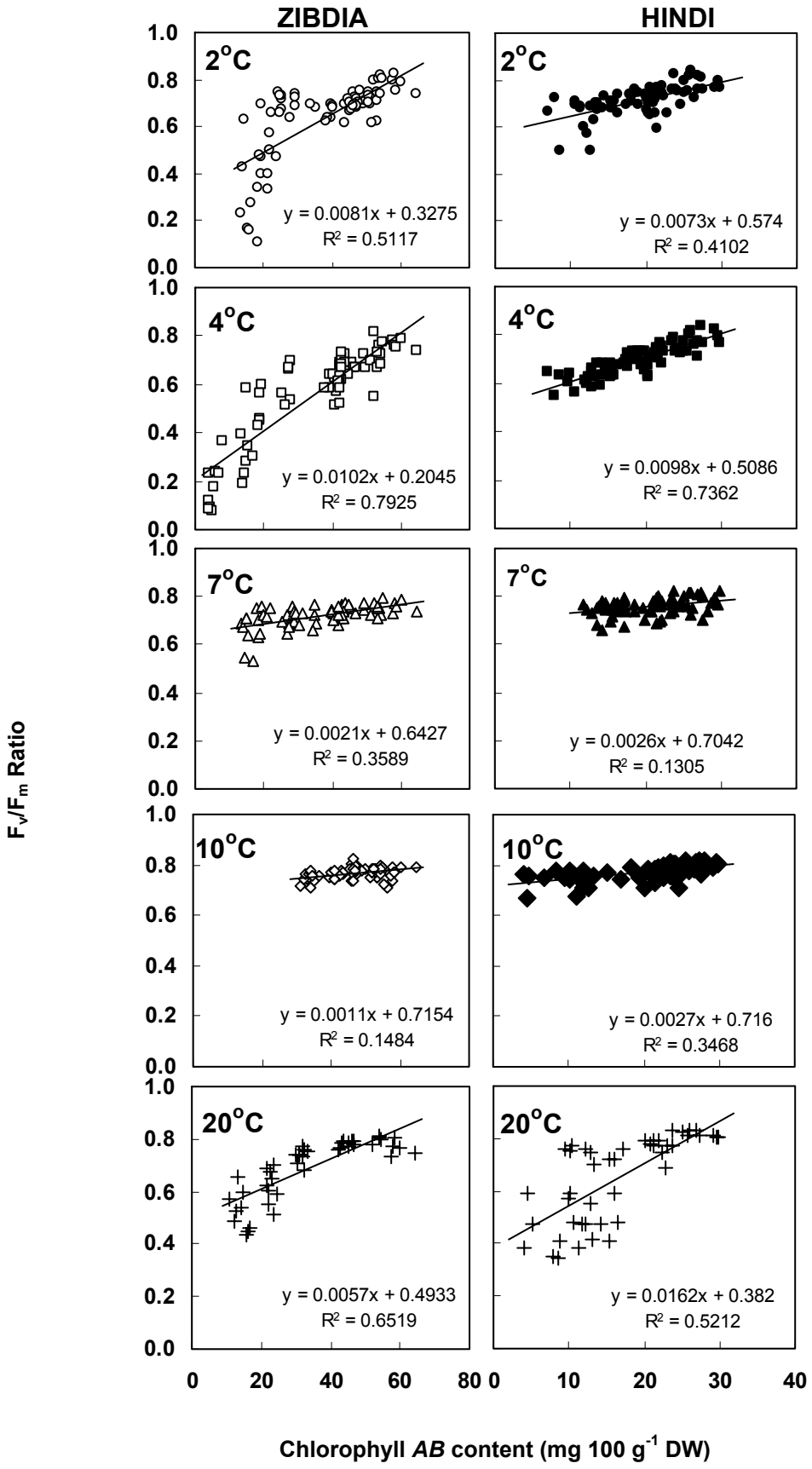

Figure 11. The correlation F_v/F_m ratio in function of total chlorophyll AB content of two mango cultivars Zibdia (A) and Hindi (B) stored at different storage temperatures for 35 days, (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10^0C and -+-) and (Hindi: - ● - 2, - ● - 4, -▲- 7, -◆- 10^0C and -+-). Solid lines represent linear regressions on the data.

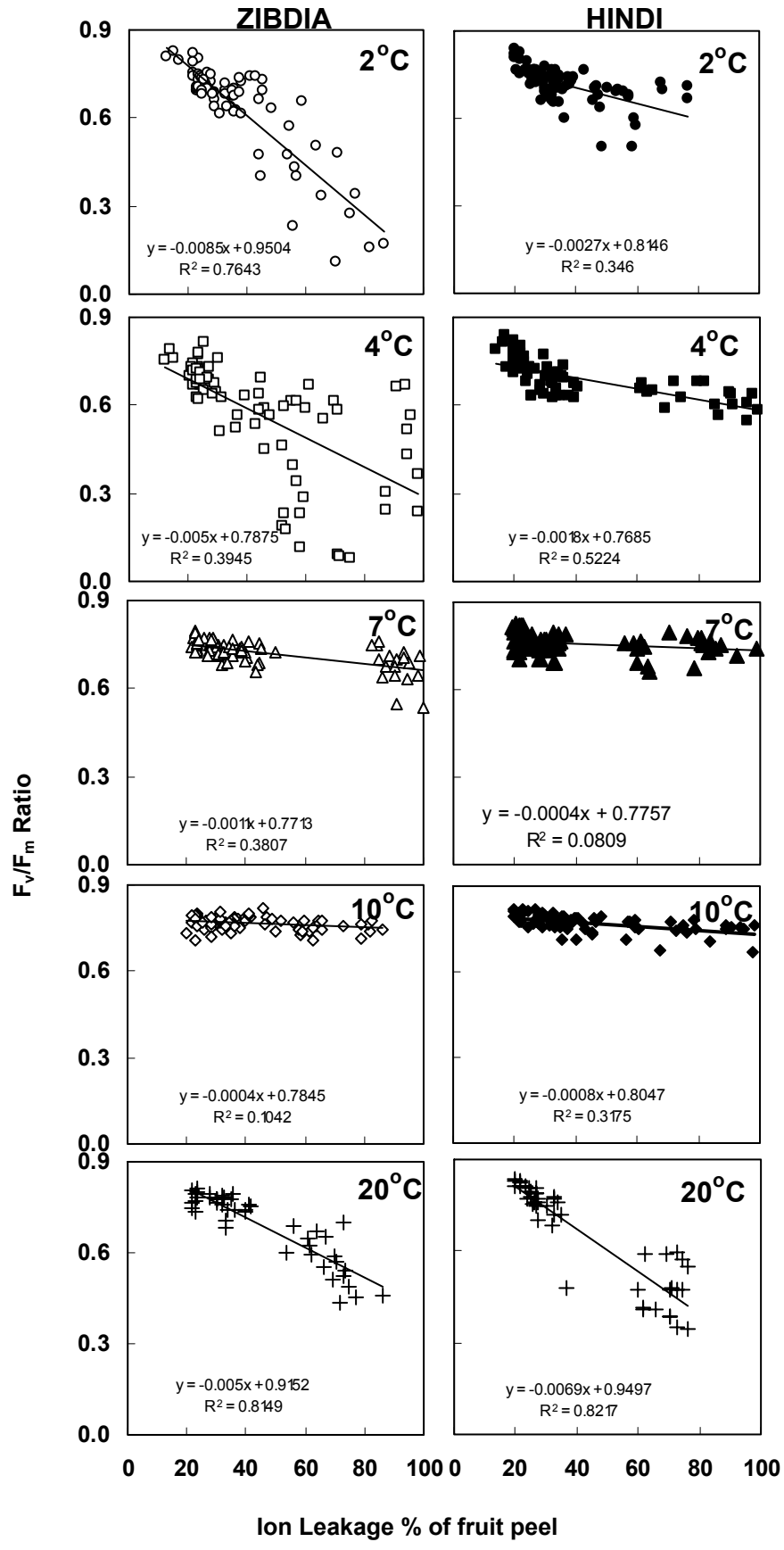

Figure 12. The correlation F_v/F_m in function of ion leakage percentage of two mango cultivars stored at different storage temperatures for 35 days. (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10^{0}C) and (Hindi: -● - 2, - ● - 4, -▲- 7, -◆- 10^{0}C). Solid lines represent linear regressions on the data.

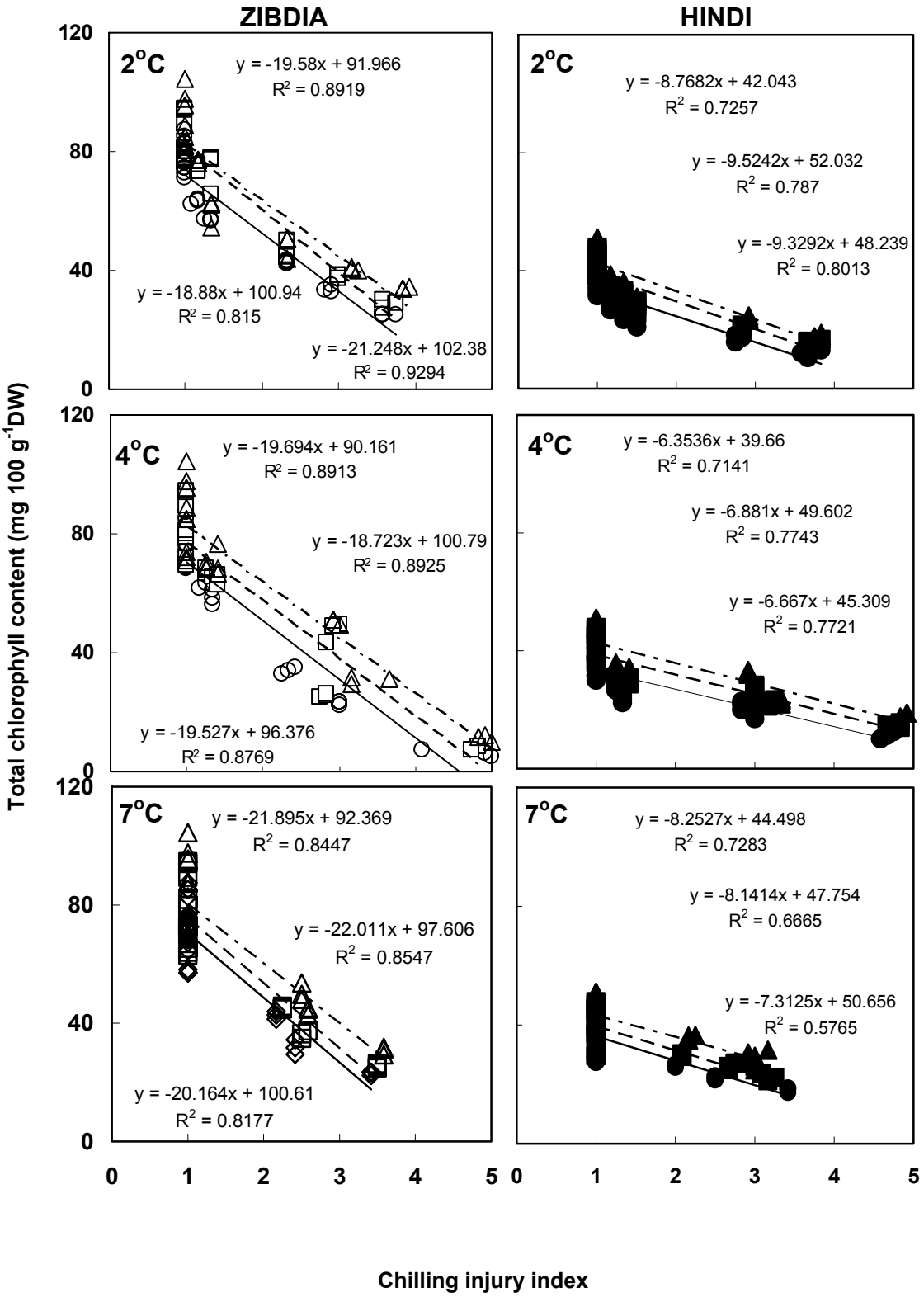

Figure 13 The correlation of total chlorophyll (A and B) content in function of chilling injury index of two mango varieties fruits of Zibdia and Hindi varieties were harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days. Solid lines represent linear regressions on the data.

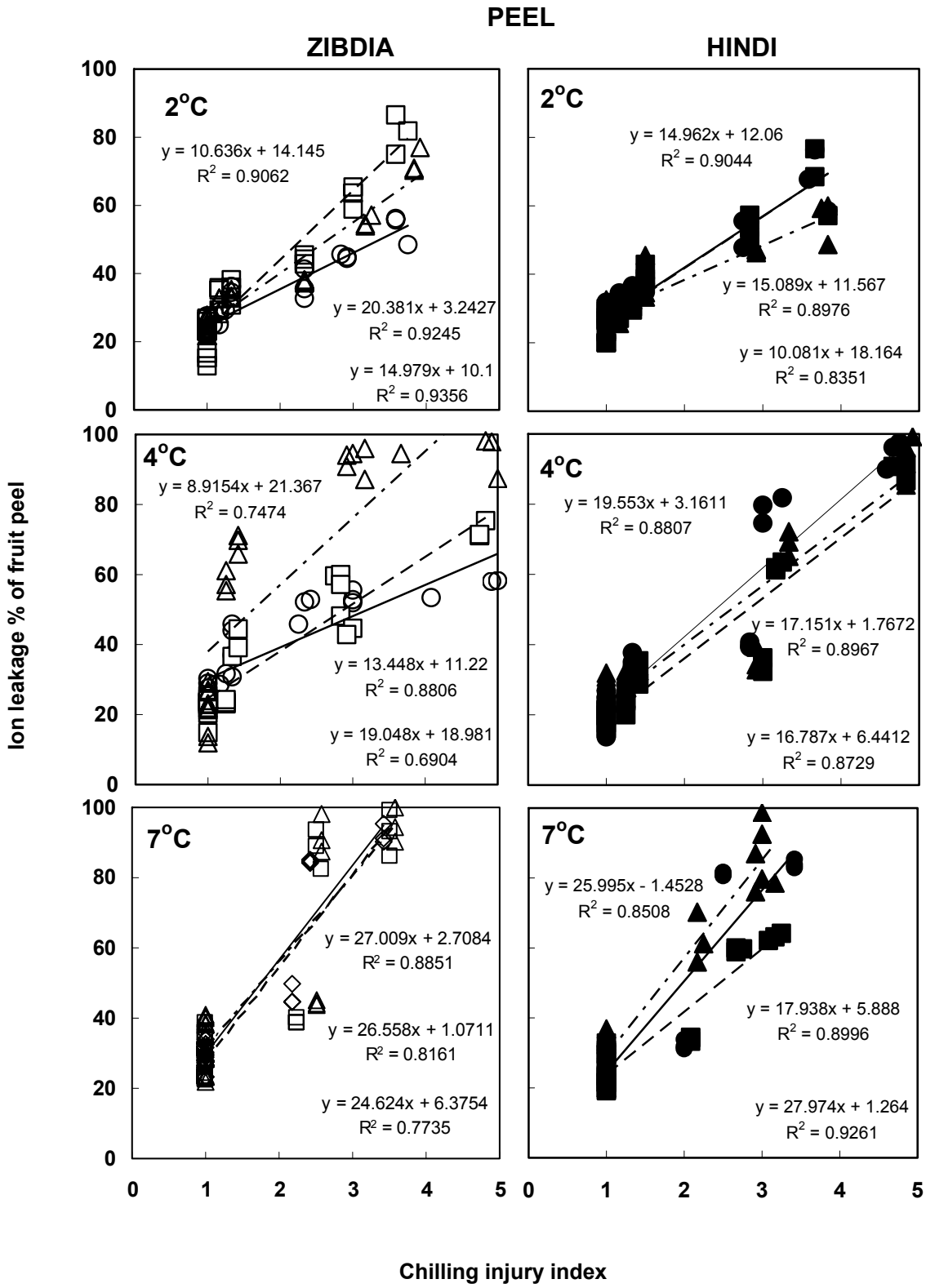

Figure 14a. Presents the linear relationship in ion leakage percentage of fruit peels in function of chilling injury index of fruits of Zibdia and Hindi varieties were harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days. Solid lines represent linear regressions on the data

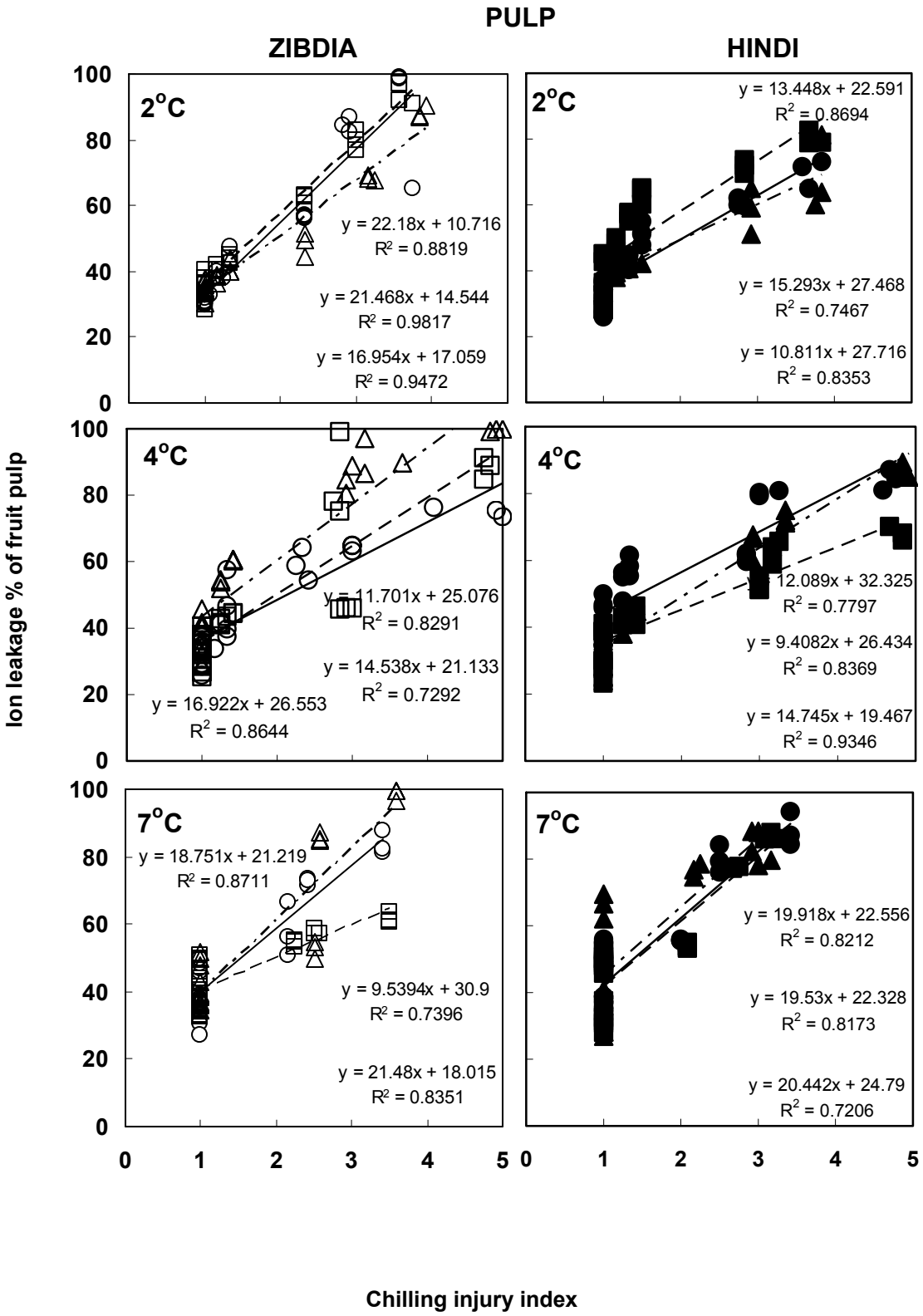

Figure14b. Presents the linear relationship in ion leakage percentage of fruit pulp in function of chilling injury index of fruits of Zibdia and Hindi varieties were harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days. Solid lines represent linear regressions on the data

Chapter 4

Changes in the concentration of water and lipid-soluble antioxidants during cold storage of Zibdia' and 'Hindi Be-Sennara' mango fruits

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Abstract

The predominant water (ascorbic acid (ASC)) and lipid soluble (α-Tocopherol (α-TOC) and β-Carotene (β-CAR)) antioxidants (WSA and LSA, respectively) of two mango varieties (*Mangifera indica* L. 'Zibdia 'and 'Hindi Be-Sennara) were measured in the peel and the pulp of fruits stored at temperatures of 2 - 20^{o}C for 35 days. The initial amounts of WSA at the time of harvest were found to differ according to the maturity stage of the fruit. The WSA content also decreased during storage dependent on maturity stage at harvest. Zibdia had a higher initial ASC content than Hindi and had a higher content in for M1 and M2 fruit relative to M3 fruits in both peel and pulp. For the LSA, the patterns of change were more complex. In the peel of Hindi the initial level of α-TOC increased with increasing maturity stage, but decreased during cold storage, and the same pattern was found in Zibdia except that the initial level of α-TOC was lower than for Hindi. In the pulp of Hindi the α-TOC increased strongly with maturity stage and decreased during storage, whereas with Zibdia the α-TOC hardly changed with maturity stage. β-CAR contents that increased by increasing fruit maturity in both Zibdia and Hindi and also increased during storage, even at 2°C. These results were pronounced even before visible CI symptoms. Storage Zibdia fruits at 10^{o}C can be considered as the optimum storage temperature for however, 7^{o}C is optimum for Hindi in terms of preserving anti-oxidant levels

Keyword: Ascorbic acid (ASC); α-Tocopherol (α-TOC); β-Carotene (β-CAR); Mango fruits

1. Introduction

Fruits and vegetables contain many different water (WSA) and lipid-soluble antioxidants (LSA), e.g. ascorbic acid (ASC), α-Tocopherol (α-TOC) and β-Carotene (β-CAR). Epidemiological studies suggest that a high consumption by humans of fruits and vegetables is associated with numerous health benefits such as a lower incidence of various degenerative diseases, reduced cardiovascular disease, reduced risk of cancer, and enhanced immune system, reduced levels of cell injury and an improved ability to detoxify contaminants and pollutants, (Ching and Mohamed, 2001; Feskanich et al., 2000; Haegele et al., 2000; Leong and Shui, 2002; Michets et al., 2000; Wang and Jiao, 2000). WSA and LSA are considered to protect tissues against active oxygen species (AOS), including superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$) and singlet oxygen ($^1O_2$) (Heinonen and Meyer, 1998; SatueGracia et al., 1997). It is thought the epidemiologically demonstrated health benefits of fruit and vegetables are due at least in part to the LSA and WSA that they contain.

Just as LSA and WSA are thought to protect against AOS in animals, then they act in the same way in plant cells where AOS are formed in different organelles (Hodges et al., 2004). Several studies have outlined the effect of excessive levels of AOS formation in the plant cell. They produce increases in oxidative reactions that result in biochemical and physiological degradation of the lipids and proteins of the cell membrane (Purvis, 2003). Practically, many storage disorders of fruits are associated with oxidative damage (Purvis, 2004).

The health benefits of humans of the naturally occurring LSA and WSA found in vegetables and fruits have led to these substances being significant determinants of the compositional quality of these products. The LSA and WSA content of a product can differ for many reasons, such as maturity stage at harvest, the variety, geographic or climatic effects, and post-harvest processing and storage conditions (Adriana and Delia, 1998). In the case of mango varieties the maturity stage of fruit at harvest is important: ASC was reported to decrease with increasing fruit maturity whereas α-TOC and β-CAR increase with increasing fruits maturity (Lee and Kader, 2000). Recently, it was reported that significant changes in α-TOC content which increased during ripening of fruits apple. For example, delayed harvesting of apple raised α-TOC content in the peel, though this decreased during postharvest processing, e.g. storage and packaging (Barden and Bramlage, 1994), The β-CAR content has also been reported to increase during the ripening and ageing of mango fruits (Exotic Fruits, 2000).

Munne-Bosch and Alegre (2002) have summarized the role that ASC plays in plant cells. It participates in a wide range of metabolic processes including

photosynthesis, photoprotection, the cell cycle, cell-wall growth expansion, synthesis of ethylene, gibberellins, anthocyanins, and hydroxyproline, and resistance to environmental stress. An important role for ASC is the detoxification of superoxide, hydrogen peroxide and singlet oxygen by means of a series of redox reactions involving which also involve glutathione (Foyer et al., 1991). α-TOC is the major lipid-soluble antioxidant in bilayer membranes, which it protects against singlet oxygen, peroxyl radicals and nitrogen oxide species (Christen et al., 1997). In this way it stabilizes the cell membrane (Shewfelt and Del Rosario, 2000) and thus prevents damage of tissues arising from membrane dysfunction (Combs, 1992). The carotenoids, in general have two main anti-oxidant roles: they can act as radical scavengers and in photosynthetic tissues they are also a quencher of triplet-excited states of chlorophyll a (Hideg et al., 1998); (Mortensen and Skibsted, 1997) Carotenoids scavenge free-radicals in two ways First they can react with radicals to form stable molecules, and second they can reduce the radical, forming a stable carotenoidic radical cation from which a carotenoid can be regenerated. The aim to this study is to measure how WSA and LSA change during storage at different temperatures in term of developmental changes of two mango varieties are harvested on different maturity stages. In addition to providing information about how storage will affect the nutritional value of the mangoes, we also intend to correlate the changes in anti-oxidant levels with the development of oxidative injury in the mango fruit and thus examine the possibility that changes in fruit anti-oxidant levels are involved in the development of the symptoms of chilling injury via increase in oxidative damage to cell lipids and protein.

2. Materials and methods

2.1. Fruits

Fruits from two mango varieties ‘Zibdia’ and ‘Hindi Be-Sennara’, were harvested, and imported from Sharqia province, East Egypt (30.35 N and 31.30 E). The harvesting and logistic chains were described before in chapter 2. day zero on the graphs was day on which the first measurement was made:

2.2. Storage conditions

The fruits were divided into 5 batches; each one of which was composed of 300 Hindi and 300 Zibdia (100 fruits of each maturity stage). These batches were stored in 5 different containers at 5 different temperatures: 2, 4, 7, 10 and 20^{o}C. Twelve fruits were collected from each container every 5 days (up to 35 days) from which samples of peel and pulp were removed and stored at –80^{o}C until required for analysis of ASC, α-TOC and β-CAR.

2.3. ASC content

To exactly an weighed 5 g sample, 5 ml of 9.5 % w/v oxalic acid, 5 ml methanol, and 35 ml Milli-Q water were added (Veltman et al., 2000). The mixture was homogenized for 1-2 min before being filtered 4°C in darkness to avoid photo-oxidation of ASC. The filtrate was injected directly into the HPLC system. The sample temperature was kept at 4°C in the automated sampler of the HPLC. The HPLC system comprised of a Dionex model P580 pump (Dionex, Sunnyvale, USA), a Lichrospher® 100 RP-18 (5 cm) Lichro CART® 250-4 column (Merck, Darmstadt, Germany) and a Dionex UVD 3405 detector (251 nm). The mobile phase used was 2.5 g tetrabutylammoniumhydrogensulfate and 55 ml methanol dissolved in 942.5 g Milli-Q water. The flow rate was kept at 1 ml min^{-1} and the analyses were completed within 15 min.

2.5. Protein carbonyl assay

Protein carbonyl grope was measured according to (Levine et al., 1994). Precisely weighed mango samples (peel or pulp) of about 2.5 g were ground in a mortar and mixed with 10 ml of 20 mM potassium phosphate buffer (pH 7.0) to extract soluble proteins. The homogenates were then centrifuged at 16000 rpm for 5 min. One ml samples of the supernatant, to which 500 μl of 10 mM 2,4-dinitrophylhydrazine in 2 M HCl had been added, were allowed to stand at room temperature for 1 hour, with vortexing every 10-15 min. 500 μl of 20% trichloroacetic acid was then added and the mixture centrifuged at 11000g for 3 min. The supernatant was removed and the precipitate washed 3 times with 1:1 v/v ethanol/ethyl acetate mixture to remove any un-reacted dinitrophenol. The sample was then allowed to stand for 10 min before centrifugation after which the supernatant was discarded. The washed precipitated protein was then allowed to dissolve in 0.6 ml guanidine solution (6 M) for 15 minutes at 37°C, after the mixture was centrifuged to remove any undissolved material. Afterwards, the spectrum was measured spectrophotochemically against the complementary blank in case of cured (without sample) samples or against water in case of purified proteins. The carbonyl content of the protein was calculated from the absorbance of the dinitrophenylhydrazone measured at 390nm and assuming an extinction coefficient of 22000 M^{-1} cm^{-1}.

2.4. α-TOC and β-CAR extraction

To avoid photooxidation of CAR the α-TOC and β-CAR were extracted in darkness and, all extracts were stored in brown bottles. The samples of peel and pulp were dehydrated at -80°C in a freeze-drier for 7 days after which they were stored in a desiccator at room temperature. To extract the α-TOC and β-CAR the

freeze-dried samples were first pulverized in a ball–mill. To 0.8 g of this powder 5 ml *N,N*-dimethylformamide (DMF) was added. The powder/DMF suspension was sonicated for 15 min at 4°C (Minguez-Mosquera et al., 1991) and then stored at 4°C for 16 hours to allow complete extraction of the α-TOC and β-CAR (Mónica et al., 1994). Finally, 1 ml of the suspension was centrifuged for 5 min at 16000 rpm at 4°C to remove all solids and the supernatant solution was then injected into the HPLC system.

2.6. Lipid peroxidation

Exactly weighed 2.5 g mango (peel or pulp) samples were ground in a mortar and mixed with 25 ml of 5% (w/v) metaphosphoric acid, 500 μl of 2% (w/v) butylated hydroxyltoluene in ethanol, and finally homogenized by a mixer. The homogenates were filtered and centrifuged at 15000 rpm for 20 min. Then chromogen was formed by mixing 1 ml of the supernatant solution, 100 μl of 2% (w/v) butylhydroxytoluene, 0.50 ml of 1% (w/v) TBA (thiobarbituric acid) in 50 mM NaOH and 0.50 ml of 25% (v/v) HCl, and incubating the reaction mixture at 95°C for 30 min. The reaction blank was prepared by replacing the sample with extraction medium and the control for each sample were prepared by replacing TBA with 50 mM NaOH. The calibration curves made by measuring 1,1,3,3-tetraethyoxypropane (Sigma) in the range 0-2 mM (TBARS) which was equivalent to 0-1 mM malondialdehyde (MDA). Tetraethyoxypropane is stoichiometrically converted into MDA during the acid-heating step of the assay (Iturbe-Ormaetxe et al., 1998). The amount of TBARS present is expressed as MDA equivalents.

2.7. Assay of protein content

The total protein content in the peel and the pulp of the mango fruits was determined using the method reported by Bradford, (1976) using bovine serum albumin as an internal standard.

2.8. Statistical analysis

The data were subjected to analysis of variances. The storage temperatures, time, varieties, and maturity stages are effect on chilling injury and water and lipids-soluble antioxidants. The interaction was assessed within the analysis of variances. The comparisons of difference means were undertaken using the least significant differences (L.S.D.) at p=0.05. Data was analyzed two periods: the first is from 0 - 20 days, when treatments are presented and the second is from day 0 up to 35 days for all treatment except for treatment at 20°C. Linear regression analysis and ANOVA were analyzed at the 5% probability level. The statistical software package Genstat 8 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) was used.

3. Results

3.1. ASC

Figure 1 shows the changes of ASC (mg per 100 g^{-1} FW) content in both mango varieties harvested at three different maturity stages as a function of storage time (days) at different storage temperatures (2, 4, 7, 10 and 20^{o}C), in the peel (a) and the pulp (b). In fact, ASC shows significant interactions ($p<0.001$), when the storage factors such as time, temperatures, varieties, and maturity stages were considered. At day zero Zibdia fruits have a three-fold higher ASC content in both the peel and pulp than Hindi. Zibdia has slightly more ASC in the pulp compared to the peel at day zero for all maturity stages, whereas in Hindi fruits at day zero the peel and pulp have similar amounts of ASC The ASC content in the peel decreases as the fruit maturity increases, though less so for Hindi than Zibdia. In the pulp of Zibdia fruits the ASC concentration at day zero is relatively unaffected by maturity stage, whereas in Hindi pulp ASC concentration decreases slightly as the fruit maturity increases. The ASC content decreases with increasing storage time for all storage temperatures and maturity stages. The decrease in ASC is greatest in the pulp of the fruits and is more pronounced in Zibdia than in Hindi fruits. At 20°C storage the loss of ASC from the pulp was rapid from both Hindi and Zibdia fruits, so that after 20 days of storage (by which time the fruits had completely ripened) the level in Hindi had decreased to close to zero for all maturity stages and in Zibdia the amount ranged from 30 mg 100 g^{-1} FW at M1 to 10 mg 100 g^{-1} FW at M3. In the peel at 20°C the loss of ASC was more gradual than in the pulp so that even though the starting value was lower than for the pulp, after 20 days of storage there was always more ASC in the peel compared to the pulp. The decrease in ASC during storage at low temperatures was relatively independent of storage temperature in Hindi fruit. In the peel the decrease is generally least for fruits stored at 7°C, and in the pulp is least for fruit stored at 10°C. These differences are, however, small and not significant. In the case of Zibdia the effect of storage temperature at low storage temperatures is more marked. In both peel and pulp and for all maturity stages 10°C storage produces the smallest decrease in ASC content, followed by 7°C, 2°C and finally 4°C. In the case of the peel, the effect of temperature on ASC content under low temperature conditions was strong. In comparison to storage at 20°C, storage at low temperatures resulted in a reduced loss of ASC except for Zibdia peel at the M1 and M2 stages were the decrease at 20°C was comparable to that at 4°C.

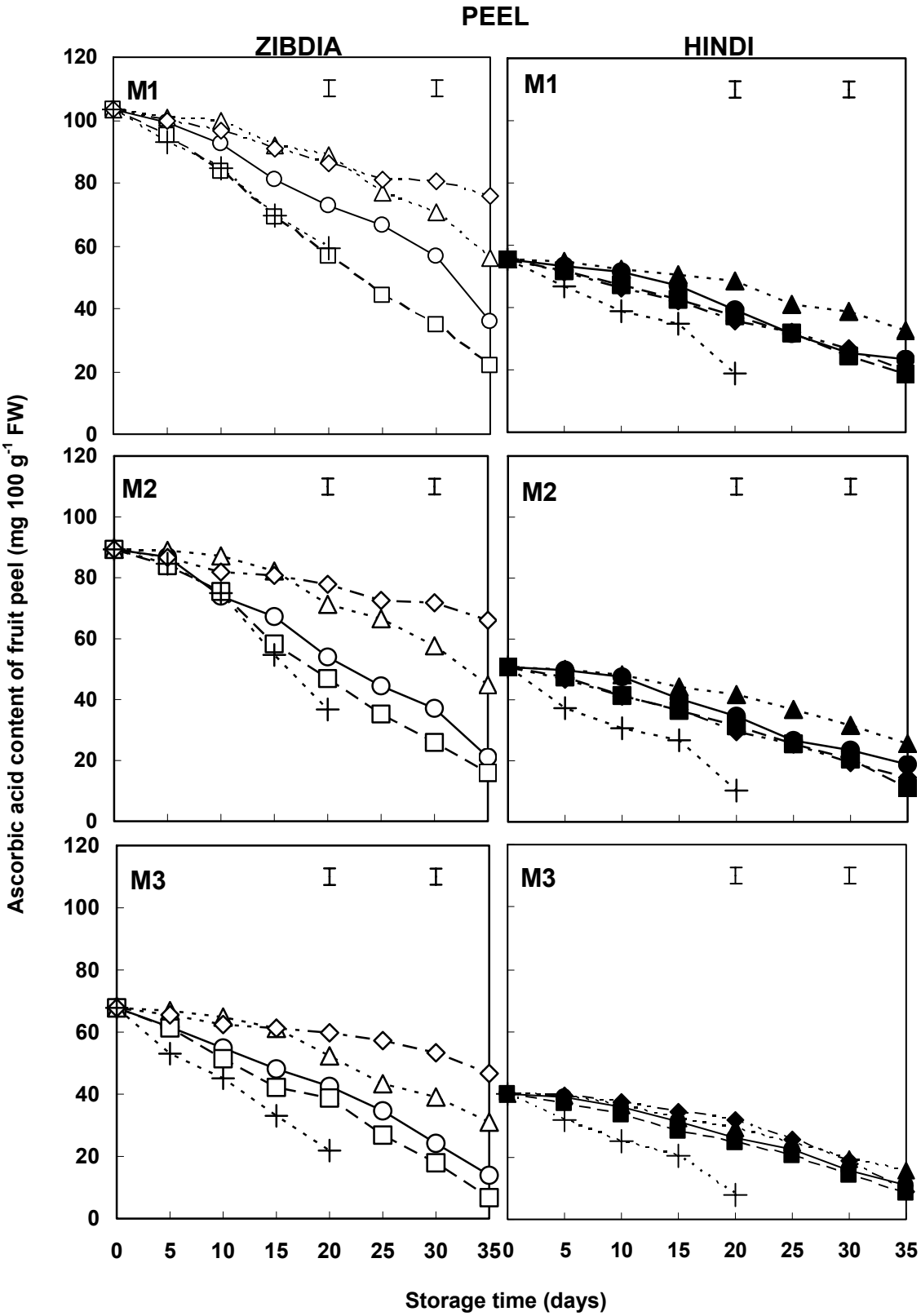

Figure 1a. Ascorbic acid content of Zibdia and Hindi varieties harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured of fruit peel versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). Ascorbic acid content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

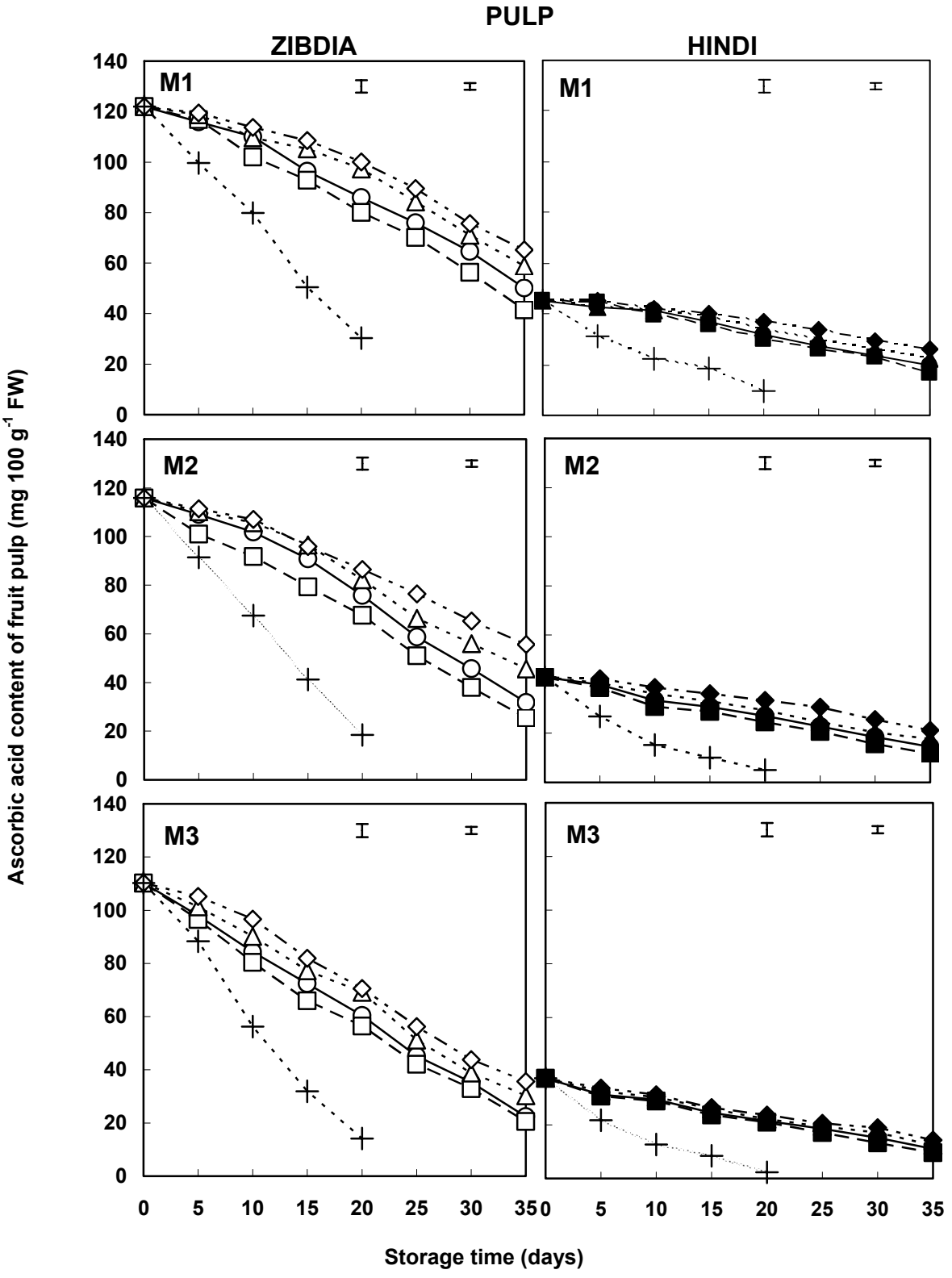

Figure 1b. Ascorbic acid content of Zibdia and Hindi varieties harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured of fruit pulp versus storage time. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). Ascorbic acid content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

3.2. α-TOC

Figure 2 shows a significant interaction (p<0.001) of α-TOC (mg per 100 g^{-1} DW) plotted as a function of storage time (days) for both mango fruits harvested at three maturity stages studied at different storage temperatures (2, 4, 7, 10 and 20°C). Compared to Zibdia a higher α-TOC content was found in Hindi at time of harvest for most maturity stages, especially in peel. Only in pulp of the M1 stage did Hindi have a lower α-TOC than did Zibdia. For instance, in Hindi peel α-TOC was found to range from 15-18 mg per 100 g^{-1} DW, whereas, it was 9-11 mg per 100 g^{-1}
DW in Zibdia peel. The α-TOC content of the peel and especially the pulp increased with fruit maturity at day zero for Hindi, whereas for Zibdia fruit maturity had only a minor effect on α-TOC content. The peel always contained more α-TOC than the pulp. At all the storage temperatures, α-TOC content decreased during storage in both mango varieties and for all maturity stages in the peel as well as in the pulp. Storage at 20°C produced the highest decay rates compared to other storage temperatures for both varieties at all the maturity stages. At this temperature there was a strong effect of maturity stage on the loss of α-TOC in both varieties: in M1 fruits the α-TOC level has decreased to almost zero by 20 days (the end of the storage period at this temperature), whereas for the M3 fruits the decrease was less severe (approximately 40% loss). Under low temperature storage the loss of α-TOC from the peel and pulp in Zibdia was least for fruits stored at 10°C irrespective of the maturity stage, though for the peel the difference between the 7°C and 10°C temperatures was minor. The greatest decreases in the α-TOC content of Zibdia under low temperature storage occurred at either 2°C or 4°C, with 4°C storage generally resulting in the greatest decrease. However these changes occurring under low temperature storage were less than those produced by storage at 20°C. For Hindi fruits the story is similar: storage at 10°C resulted in the smallest decrease in α-TOC, except for M2 and M3 pulp where 7°C storage resulted in a minor improvement in α-TOC retention compared to 10°C, and 4°C storage was resulted in either the greatest loss of α-TOC or a loss comparable to that measured in 2°C.

3.3. β-CAR

The β-CAR content in the peel and the pulp of Zibdia at day zero was found to be higher than that of Hindi, and the β-CAR content in both varieties increased with increasing maturity stage. For example, the initial β-CAR content in Zibdia peel

were 4.39, 5.98, and 6.98 mg per 100 g^{-1} DW compared with Hindi peel, which contained 2.34, 3.75 and 5.15 mg per 100 g^{-1} DW at M1, M2 and M3, respectively. In the fruit pulp the β-CAR contents were 6.70, 10.64, and 13.74 mg per 100 g^{-1} DW in Zibdia whereas in Hindi there were no differences among fruit maturity stages (approximately 13.5 mg per 100 g^{-1} DW). The changes in β-CAR during storage are complicated owing to the continued synthesis of β-CAR that occurred for a limited period at all temperatures. This produced an increase in β-CAR which was then followed by a decrease in. For fruits stored at 20°C the maximum β-CAR content was reached at either 10 days (peel of all maturity stages for both varieties) or 10 or 15 days (pulp). At low storage temperatures the maximum β-CAR content in the peel occurred at 15 or 20 days and for the pulp at 15 days. In the case of Hindi pulp the time course of β-CAR content was relatively independent of maturity stage or storage temperature. For Hindi peel and the peel and pulp of Zibdia, the time course of β-CAR content was affected by storage temperature, though not much by maturity stage. In these tissues greatest levels of β-CAR were achieved when the fruits were stored at 20°C (except in the pulp of M1 of Zibdia). In low temperature storage, tissues other than pulp from Hindi developed the greatest concentrations of β-CAR at 10°C and the lowest at 4°C; storage at 2°C resulted in more β-CAR accumulation, though this sometimes took longer to reach a maximum value (Hindi peel at all maturity stages, Zibdia peel at M2 and M3 stages). Once the maximum β-CAR had been reached, the levels declined until the end of the storage period. In most cases the β-CAR declined to very low levels; sometimes the β-CAR at after 35 days storage was less than 20% that at the maximum.

3.4. Protein oxidation

Figure 4 depicts the variation of protein carbonyl (PCG) content as a function of storage time (days) at the three maturity stages for two types of mango fruits stored at the temperature range (2-20°C) in both the peel (a) and the pulp (b) of the fruits. In fact, the protein carbonyl content shows a significant interaction at $p<0.001$ when the storage factors such as time, temperatures, varieties, and maturity stages were considered. All the maturity stages of both varieties have very low initial levels (≈zero) protein carbonyl content (PCG) in both the peel and pulp parts of the fruits. At low storage temperatures the PCG content of the peel part of both varieties increases more or less linearly with storage time up to about 20 – 25 days of storage after which a large increase in PCG occurred in some tissue/temperature accumulations.

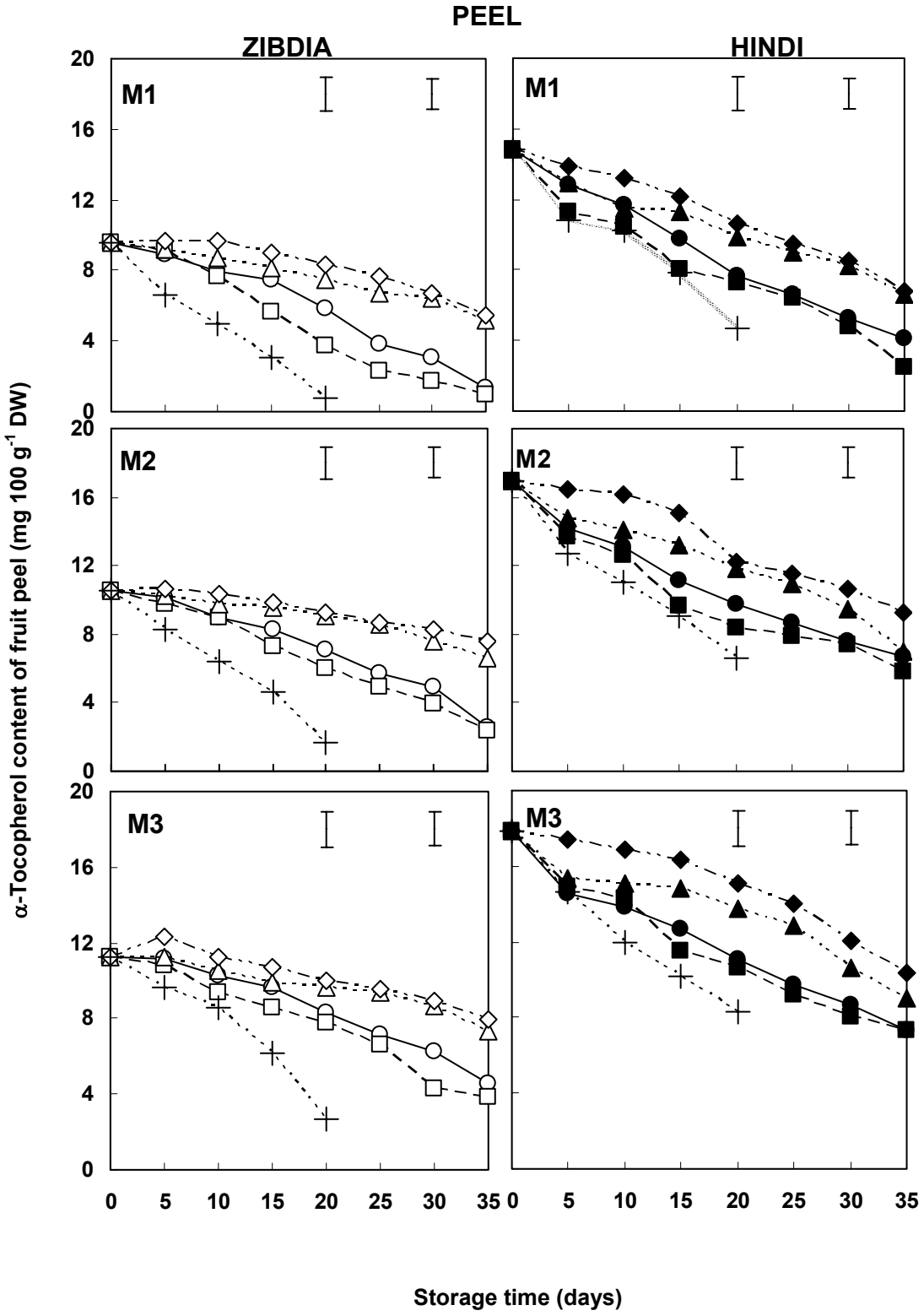

Figure 2a. α-Tocopherol content of Zibdia and Hindi varieties harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured of fruit peel, versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). α-Tocopherol content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

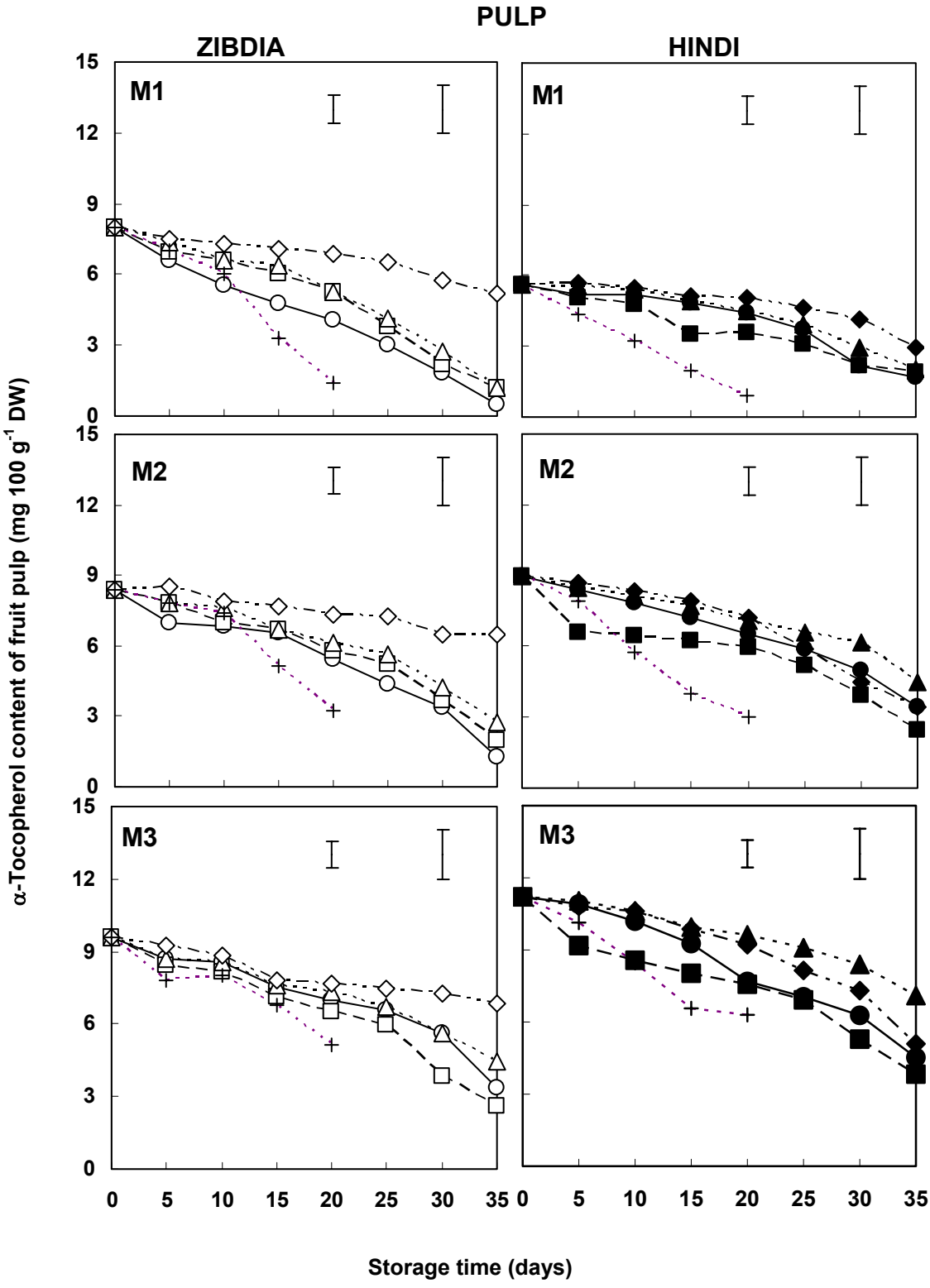

Figure2b. α-Tocopherol content of Zibdia and Hindi varieties harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured of fruit pulp versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■- 4, -▲- 7, -◆- 10 and -+-20^{0}C). α-Tocopherol content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

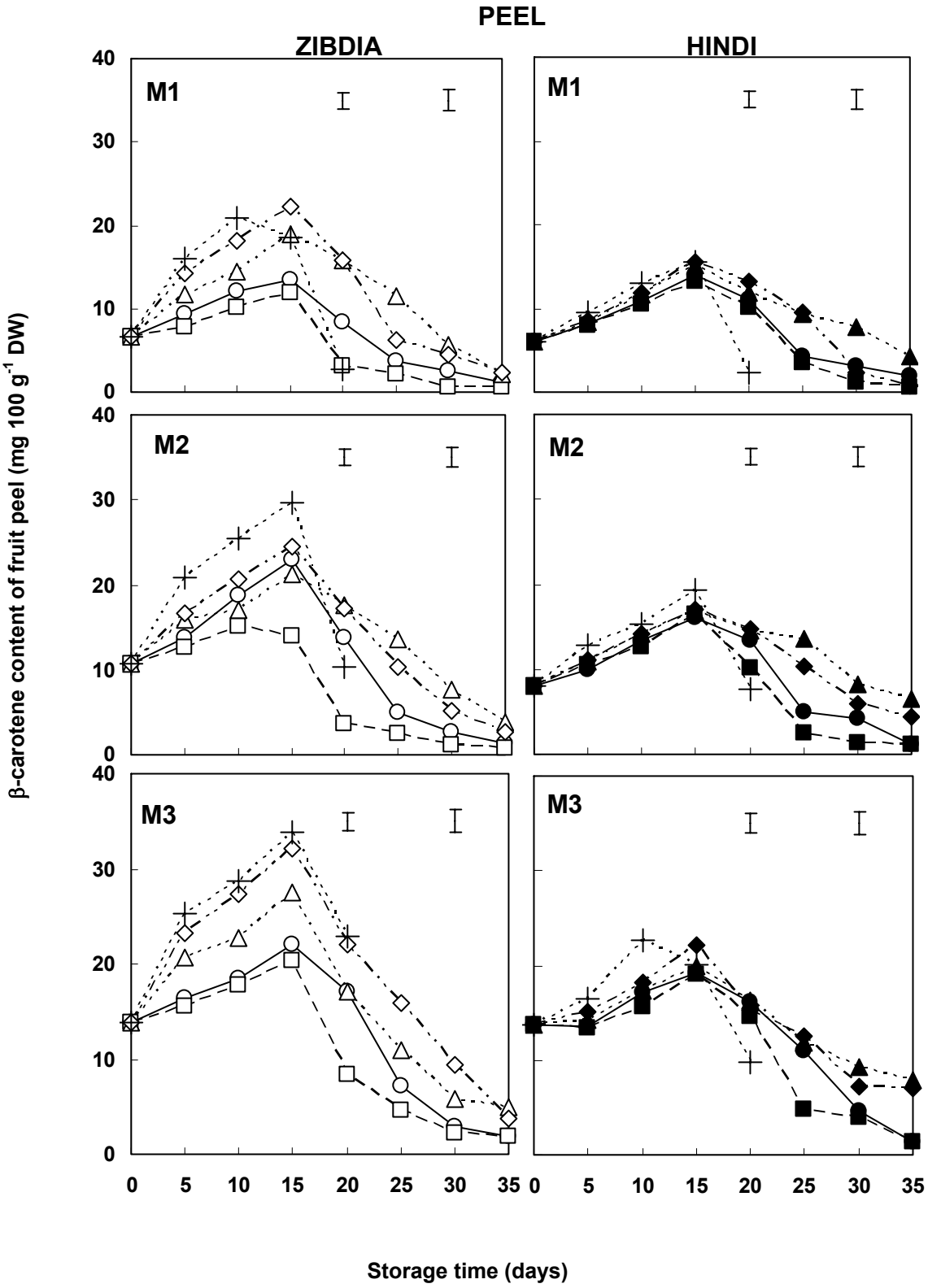

Figure 3a. β-Carotene content of Zibdia and Hindi varieties harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured of fruit peel versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). β-Carotene content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

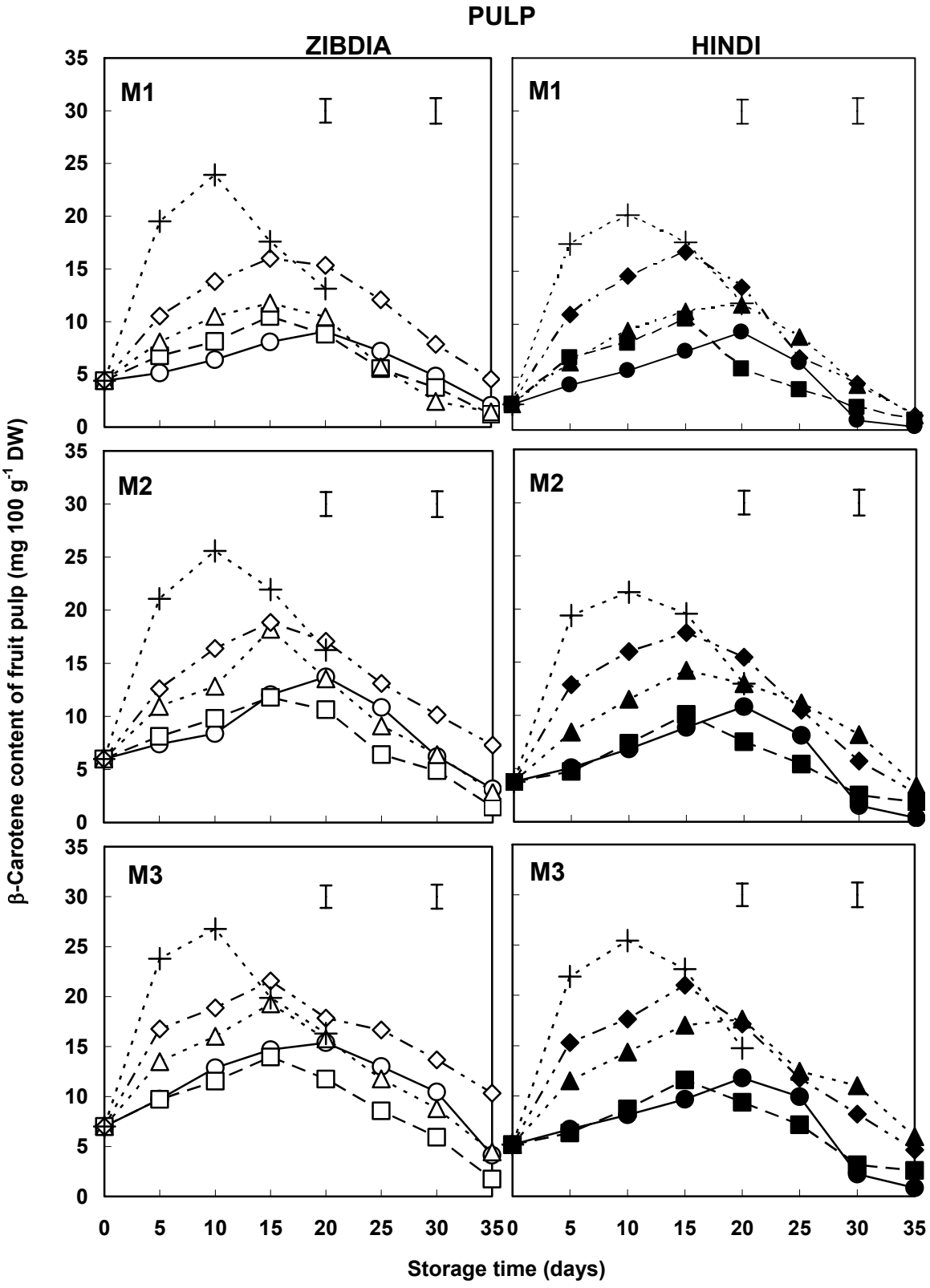

Figure 3b. β-Carotene content of Zibdia and Hindi varieties harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured of fruit pulp versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). β-Carotene content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

This increase is greatest for older maturity stages and increases as the temperature is reduced from 10°C to 4°C. Decreasing the temperature from 4°C to 2°C results in reduced increases of PCG to a level intermediate between that produced by 4°C and 7°C storage. The same behavior can be seen in fruit pulp samples though the increase in PCG content that develops after 20 – 25 days of storage is smaller than that found in peel, and in one case (Zibdia M3) the PCG accumulation at 2°C is greater than that developed at 4°C. At 20°C storage PCG levels increase rapidly in a comparable way in the peel and pulp of both fruits after about 5 days of storage. Because of the increase in the peel PCG content that develops after 20 – 25 days, PCG levels that develop at 20°C are lower than those the develop in the peel, but much higher than those that develop in the pulp i.e. the potential for PCG formation is similar in peel and pulp, but the realized PCG formation under low temperatures is less in the pulp than the peel.

3.5. Lipid peroxidation

Figure 5 shows the changes of lipid peroxidation, expressed as the concentration of malondialdehyde equivalent (MDA), in both mango varieties harvested at three different maturity stages (M1, M2 and M3) as a function of storage time (days) at different storage temperatures (2, 4, 7, 10 and 20^{o}C) in both the peel (a) and pulp (b) parts of the same fruits. In fact, the MDA shows a significant interaction at p<0.001 when the storage factors such as time, temperatures, varieties, and maturity stages were considered. The initial concentration is higher in Hindi fruits than in Zibdia fruits in the M2 and M3 stages owing to an increase in the MDA with maturity in Hindi fruits. In both fruit types, MDA concentration increases during the storage of fruits, Considering first changes in fruits stored at low temperatures, in the peel and the pulp either the rate of MDA accumulation increased as the storage temperature was decreased from 10°C to 4°C or the accumulation was unaffected by temperature in the range 10°C - 4°C. The only exception to this pattern was found in the peel of Zibdia at for the M2 maturity stage in which storage at 4°C and 10°C produced a similar degree of MDA accumulation which was higher than that found at 7°C. The behaviour of fruits stored at 2°C was unusual: for both the peel of Hindi and the pulp of Zibdia at all maturity stages, and the pulp of Hindi at M1 and M2 stages, there was an increase of MDA during storage that was greater than that occurring at 4°C. Though this pattern was also found in Zibdia peel in the M1 stage, in Zibdia peel in the M2 and M3 stages the increase at 2°C was the lower than for any other temperature. Storage at 20^{o}C caused a rapid increase in the MDA of the peel of both Zibdia and

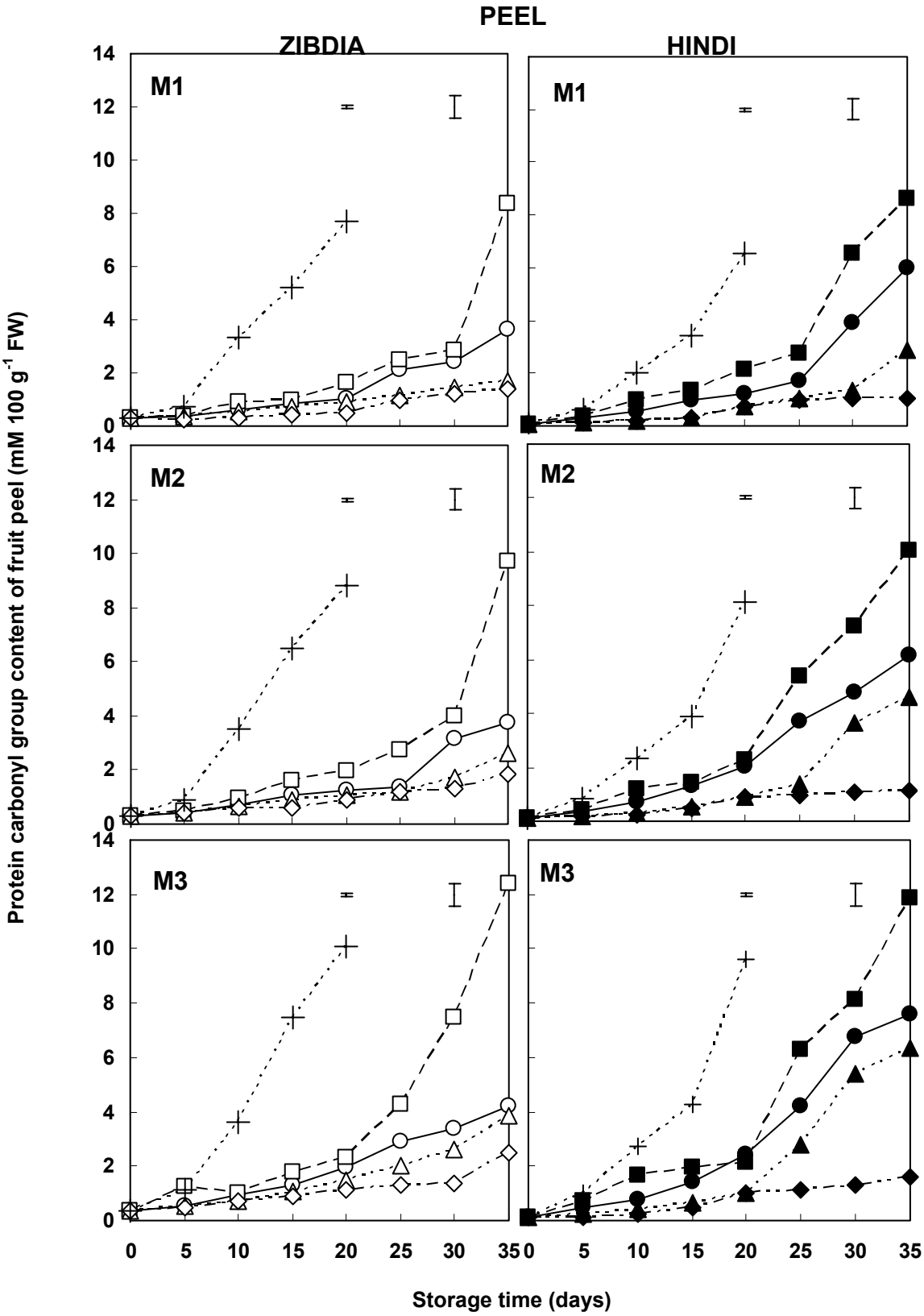

Figure 4a. Protein carbonyl group (PCG) content of Zibdia and Hindi varieties harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured on fruit peel, versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). PCG content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

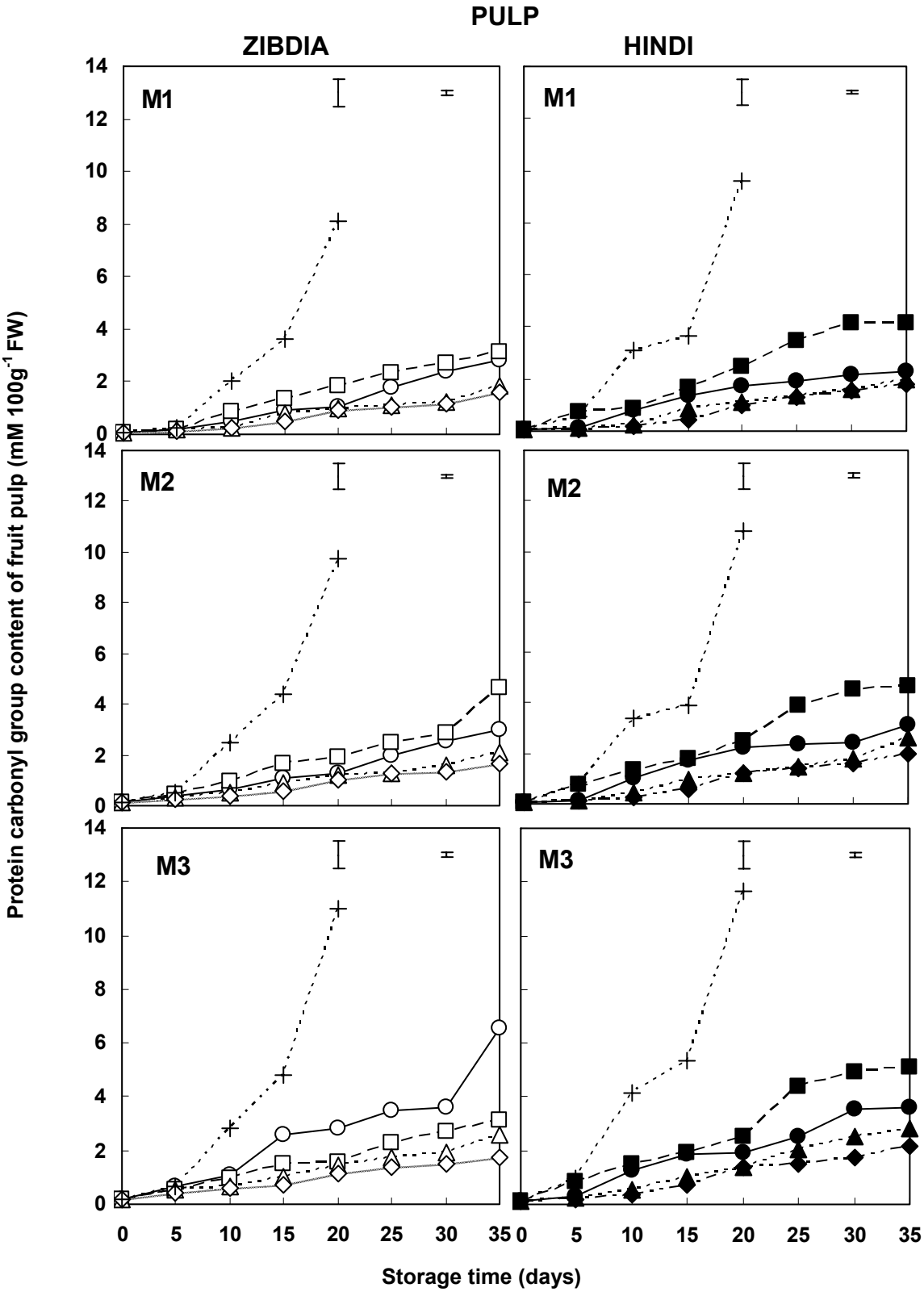

Figure 4b. Protein carbonyl group (PCG) content of Zibdia and Hindi varieties harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured on pulps versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, - ▲- 7, -◆- 10 and -+-20^{0}C). PCG content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

Hindi to a maximum level after 20 days of storage, and this increase became greater as the maturity stage at harvest increased. A similar response was found the pulp of both varieties except that in general the accumulation of MDA was less than found in the peel.

4. Discussion

One goal of storage is to maintain the levels of nutritionally significant substances, such as vitamins. In respect of vitamins in mango, only the vitamin content of the pulp is important; mango peel is not commonly included in processed mango products, nor is it eaten when the fruit is consumed directly but the condition of the peel is a fruit quality parameter. So, if changes in antioxidant content can be related to deterioration of the peel, this would be important in explaining quality changes. The differences in pulp contents response of ascorbic acid (vitamin C), tocopherol (vitamin E) and β-carotene (provitamin A) to maturation and storage are important with respect the optimization of mango as a vitamin source. Zibdia is clearly a much richer (three-fold) source of ascorbic acid than is Hindi (Ibrahim and Khalif, 1999), though ascorbic acid levels are not significantly influenced by maturity stage at harvest. This implies that there is variability, and probably genetic variability, for ascorbic acid content. This suggests the possibility of selecting or breeding cultivars with increased ascorbic acid levels. With regards tocopherol in the pulp, a different pattern of change was identified. For Hindi tocopherol content was strongly dependent on maturity stage (Barden and Bramlage, 1994), implying that for this cultivar careful control at the time of harvest is necessary if the tocopherol content is to maximized. In contrast, in Zibdia the tocopherol content is nearly as high as the maximum found for Hindi, but it is independent of maturation stage. This stability is clearly an attractive feature of this cultivar as it removes the need to be selective at harvest. The β-carotene content of Zibdia and Hindi pulp was similar, and increased with maturity stage, but given the variability observed in the amounts of ascorbic acid and tocopherol present at harvest, other cultivars may have different patterns of β-carotene concentration change during ripening may exist (Lee and Kader, 2000). These differing patterns of change with maturity stage present opportunities for both the grower and the breeder to improve the vitamin content of these fruits. Greater stability of concentration at time of harvest reduces the need for product selection during the harvesting process, and increased content at time of harvest would increase the nutritional value of the fruit.

The changes in the concentration of ASC, α-TOC and β-CAR occurring post-harvest during storage will partly determine the quality of the quality with regards to the chain. It is evident that the data the post-harvest changes in these compounds

are significant, and that they respond differently to storage; ASC and α-TOC respond in a similar way, whereas β- CAR behaves quite differently. During storage the levels of ASC and α-TOC decrease in both the peel and the pulp (Figure 1 and 2). Storage to minimize the loss these antioxidants should be at 10°C, but ideally the fruit should be used as quickly as possible after harvest. Interestingly, in the peel ascorbic acid levels decreased less than those in the pulp, so in principle it should be possible to improve the performance of pulp. In general storage produced an increase in the β-CAR level during the early period of storage, which was later followed by a decrease as storage duration increased (Figure 3). The increase in β-CAR implies that its synthesis continued, and as the increase occurred even at 2°C this requires that even in this chilling sensitive fruit ordered metabolism can still occur. It also implies that short-term storage of 10 -15 days could be used to increase the β-CAR content of the fruit, especially if the fruits are stored at 10°C or 20°C. For applications where pulp colour is important, such as juice extraction, this increase in β-CAR level might be valuable (Kader, 2002). As with other responses, there are differences in the responses of the two cultivars with regards to their β-carotene responses, which suggests the existence of genetic variability that could be exploited: the peel of Hindi and the pulp and peel of Zibdia are quite similar, whereas Hindi pulp has a smaller increase in β-carotene compared to Hindi peel.

Injury due to exposure of plant tissues to chilling temperatures has been frequently attributed to oxidative stress (Hodges et al., 2004), and oxidative stress is a consequence of the balance between the formation of AOS and their destruction shifting to favour an increased steady-state AOS level (Hodges, 2003). The formation of both the MDA equivalents and protein carbonyls are due to oxidative processes (Hodges, 2003), so it would be expected that they might be correlated. It is important to note that protein carbonyls can be formed as a result of reactions between proteins and membrane breakdown products (TBARS) as well as being initiated by hydroxyl ion attack on proteins (Berlett and Stadtman, 1997). In pulp from both Zibdia and Hindi at all maturity stages under low temperature storage the correlation is good and similar over the temperature range 2°C - 4°C. At 20°C the correlation is also good and similar for both Zibdia and Hindi, but in this case the correlation differs substantially from that found at low temperatures: large increases in PCG occur with only small increases in MDA equivalents (Figures 4 and 5). The relationship between PCG accumulation and the accumulation of MDA equivalents in peel tissues is much more variable than that found in the pulp, though it appears to be largely independent of maturity stage. For both Zibdia and Hindi the responses at 10°C and 20°C are similar.

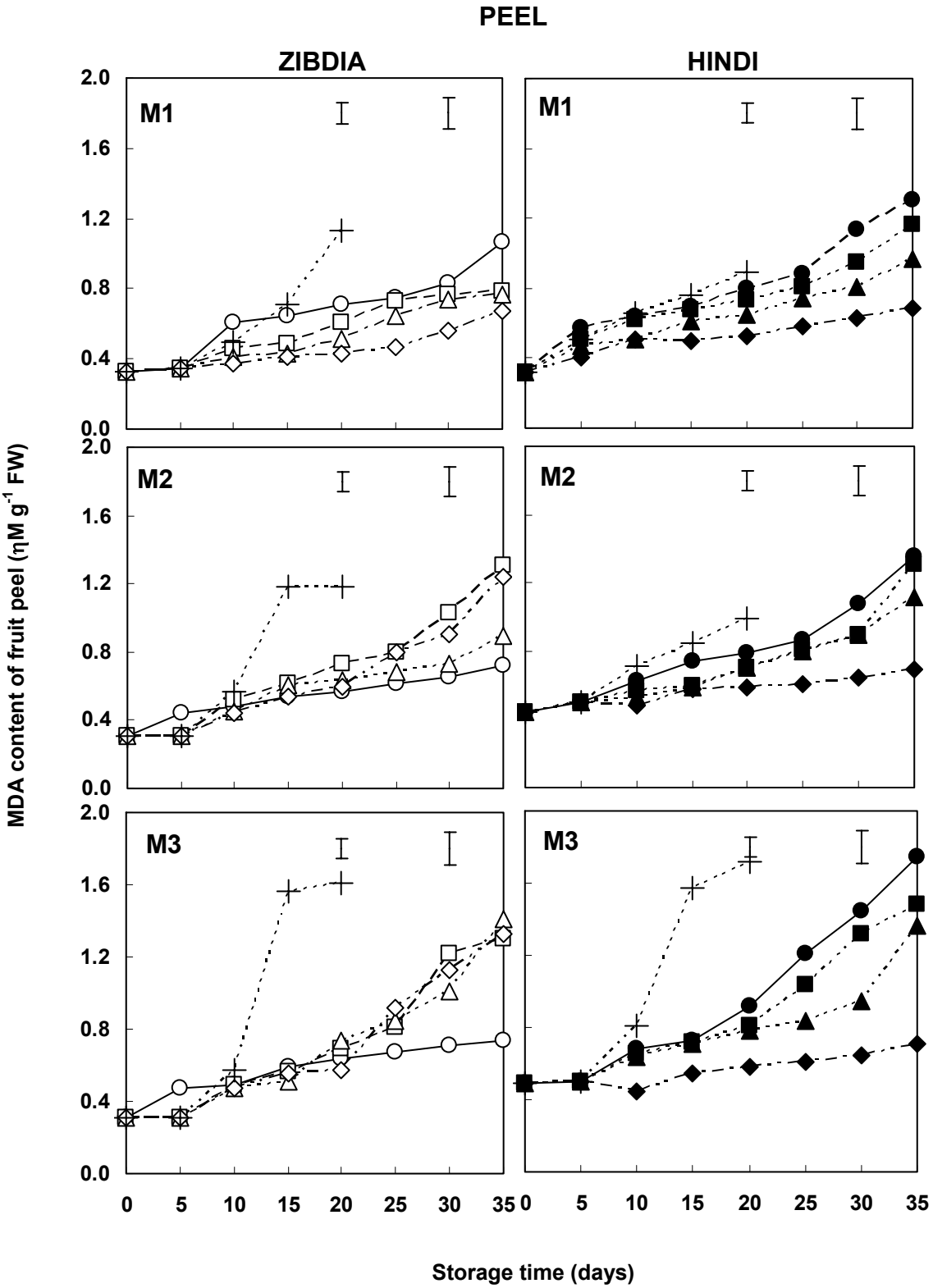

Figure 5a. Malondialdehyde (MDA) content of Zibdia (A) and Hindi (B) cultivars harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured of fruit peel stored at versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). MDA content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

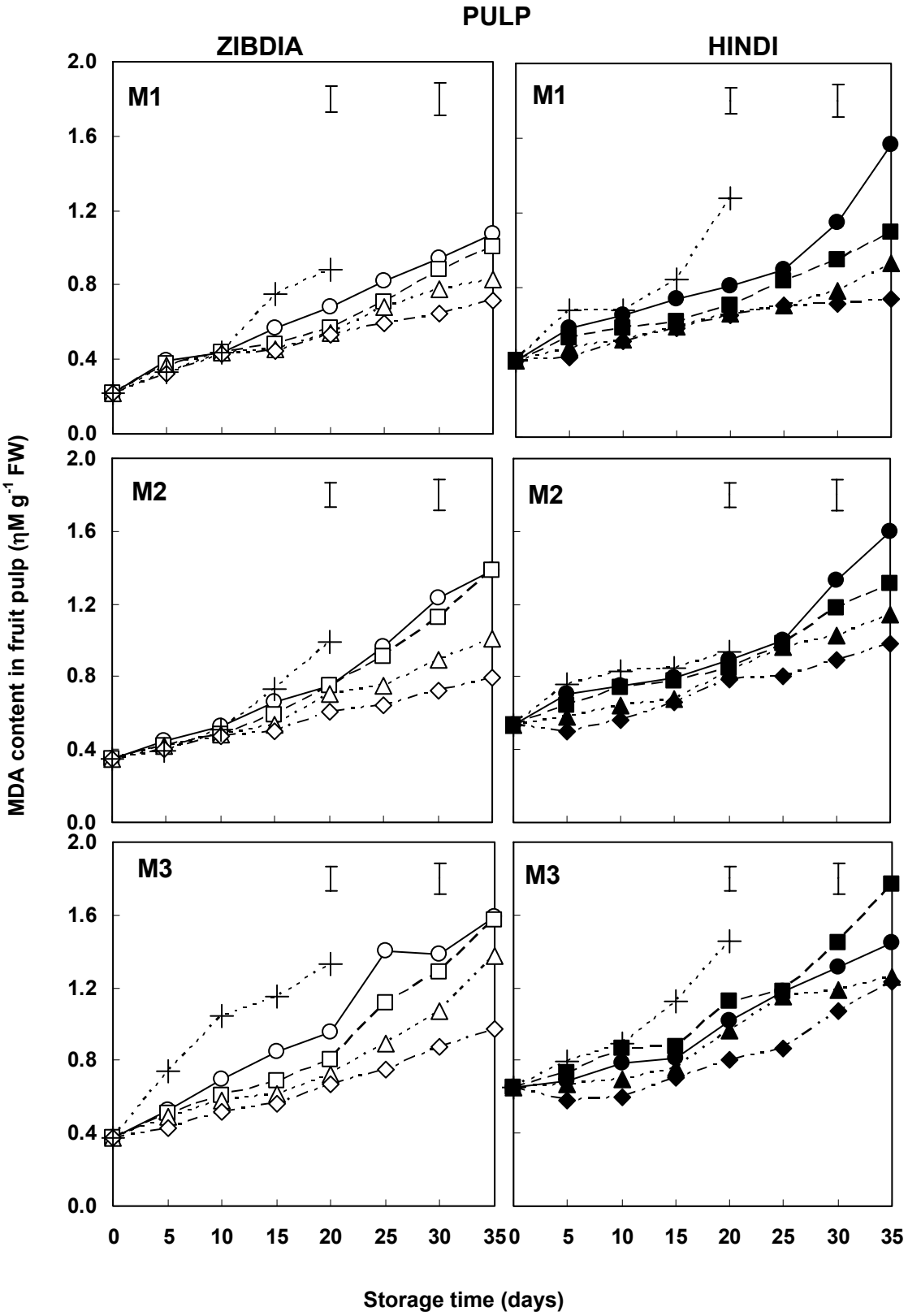

Figure 5b. Malondialdehyde (MDA) content of Zibdia and Hindi varieties harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured of fruit pulp stored versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: -●- 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). MDA content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

At lower storage temperatures there are differences between the cultivars: with decreasing temperatures Hindi has a greater formation of PCG relative to MDA equivalents, whereas with Zibdia the relative accumulation of PCG and MDA equivalents is relatively unaffected by temperature.

Taken as a whole these results show that oxidative damage is not a unitary process. Though the overall trend of oxidation is the same, the degree to which different cell components can be affected differs. It seems plausible that these differences are due to either differences in the spectrum of oxidants produced, the activity of scavenging and repair processes, or both. Protein carbonyls are a consequence of both hydroxyl radical attack and reactions with membrane breakdown products, whereas lipid oxidation is more likely to be the product of a chain reaction involves molecular oxygen, but this process is dependent upon an initiation event due to an attack by either a hydroxyl radical, or some other radical (including molecular oxygen) (Baier and Dietz, 1999). In spite of these complications, the data do show that for specific combinations of tissue, temperature and cultivar the relationship between the different measures of oxidation is generally very close, suggesting that though there are can be variations in the degree to which cell components show net oxidation, there is an underlying correlation in the oxidation process of these components.

Oxidative stress is a consequence of the balance between the formation of AOS and their destruction shifting to favour an increased steady-state AOS level (Foyer and Noctor, 2000). This could be due to either more AOS formation, or less AOS destruction. The anti-oxidants measured are involved in the elimination of AOS, but do changes in their concentration correlate with senescence or chilling related injuries to the fruits, either in the pulp or the peel. It is clear that under low temperature storage MDA accumulation, an indicator of membrane oxidation (Figure 4), correlates well with electrolyte leakage (Figure 6), which is an indicator of membrane damage. The relationship between MDA accumulation and electrolyte leakage in the pulp is similar for both varieties in the temperature range 4 -10°C. At 2°C and especially at 20°C the increase in electrolyte leakage is relatively more independent of MDA accumulation. It is possible, however, that at 20°C the rate of disappearance of MDA from the senescing tissues is comparable to the rate of its formation, thus masking an underlying correlation between membrane lipid oxidation and electrolyte leakage. So, membrane damage does seem to correlate with membrane oxidation, though in some cases this correlation is better than in others.

In relation to increase of chilling injury index during cold storage, the changes in antioxidant content in the peel and pulp are revealing. The chilling injury index is measured by observation of the peel (chapter 3), and thus need not

correlate with processes occurring in the pulp. Nonetheless, as the chilling-injury index is such a simple means with which assess injury, any correlations that may exist between peel injury and pulp injury are important to know. At temperatures where an increase in the chilling-index occurs, all the antioxidants have a broadly linear correlation between the antioxidant concentration in both pulp and peel and the CI-index. These relationships were also largely independent of storage temperature and maturity stage. Zibdia has a much higher ascorbate level in its peel than Hindi, yet the Zibdia is more chilling sensitive than Hindi and the increased ascorbate in Zibdia only results in CI-index increasing at higher ascorbate levels compared to Hindi (Figure 7).

In the case of tocopherol the situation is reversed; the peel of Hindi has more tocopherol than Zibdia, and this does seem to be associated with a smaller increase in CI-index in Hindi compared to Zibdia: this is consistent with the greater chilling tolerance of Hindi compared to Zibdia (Figure 8).

The β-carotene -CI-index correlation is similar for both cultivars. The β-carotene, concentration of the fruits has a more complicated relationship with storage-time than the other antioxidant. The increase in β-carotene concentration precedes the increase in CI-index, and it is the later decrease in β-carotene content that correlates with the rise in CI-index (Figure 9).

Given the stability of these correlations with temperature and maturity stage it could be argued that the correlation was more than coincidence and that the increase in CI-index was mechanistically linked to the decrease in antioxidant levels in the peel, and less directly to those of the pulp. However, considering only the peel data, where any mechanistic correlation between antioxidant content and CI-index would be most direct, a comparison of the relationship between antioxidant content and CI-index between the two cultivars reveals inconsistencies**.** First, at 10°C no increase in CI-index occurs, yet all anti-oxidants undergo changes in concentration that are as large as those associated with increases in chilling-injury index at lower temperatures (Chapter 3).

The chilling injury index is based upon an assessment of skin damage associated with low temperature exposure. The physiology underlying this damage is not specified, so it is possible that the link with oxidative stress is indirect. Fatty-acid oxidation, measured by means of the formation of MDA equivalents (Figure 5), is directly linked to oxidation and thus might be expected to display a better correlation with antioxidant levels. In the case of ascorbic acid in the peel, the increase of MDA is well correlated with the loss of ascorbic acid, except at 2°C where MDA levels remain low even though the ascorbic acid pool decreases almost to zero (Figure 10).

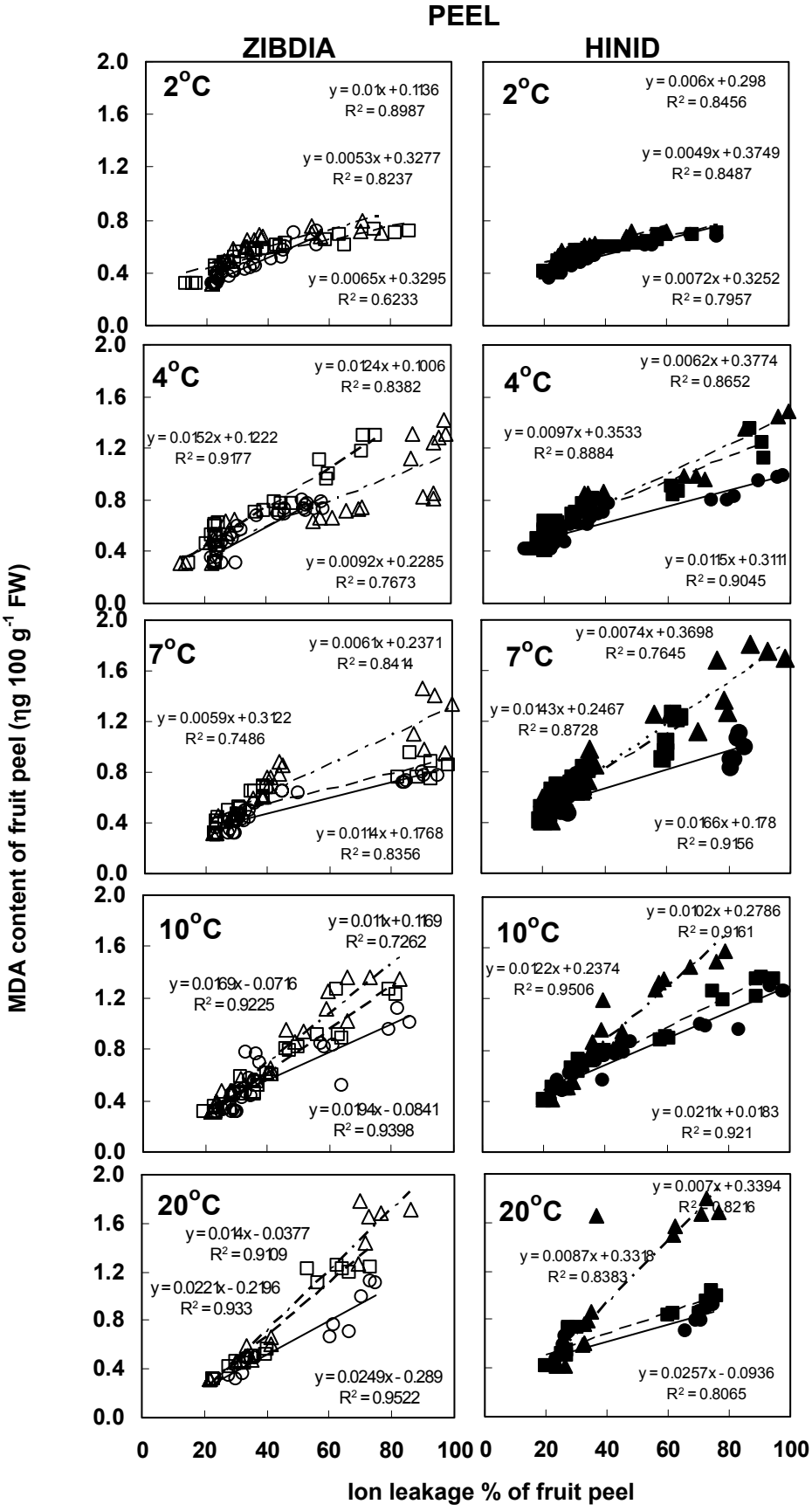

Figure 6a. The correlation between lipid peroxidation (MDA) in function α-Tocopherol content of fruit peel of two mango varieties which stored at different storage temperatures for 35 days, (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+- 20^{0}C). Solid lines represent linear regressions.

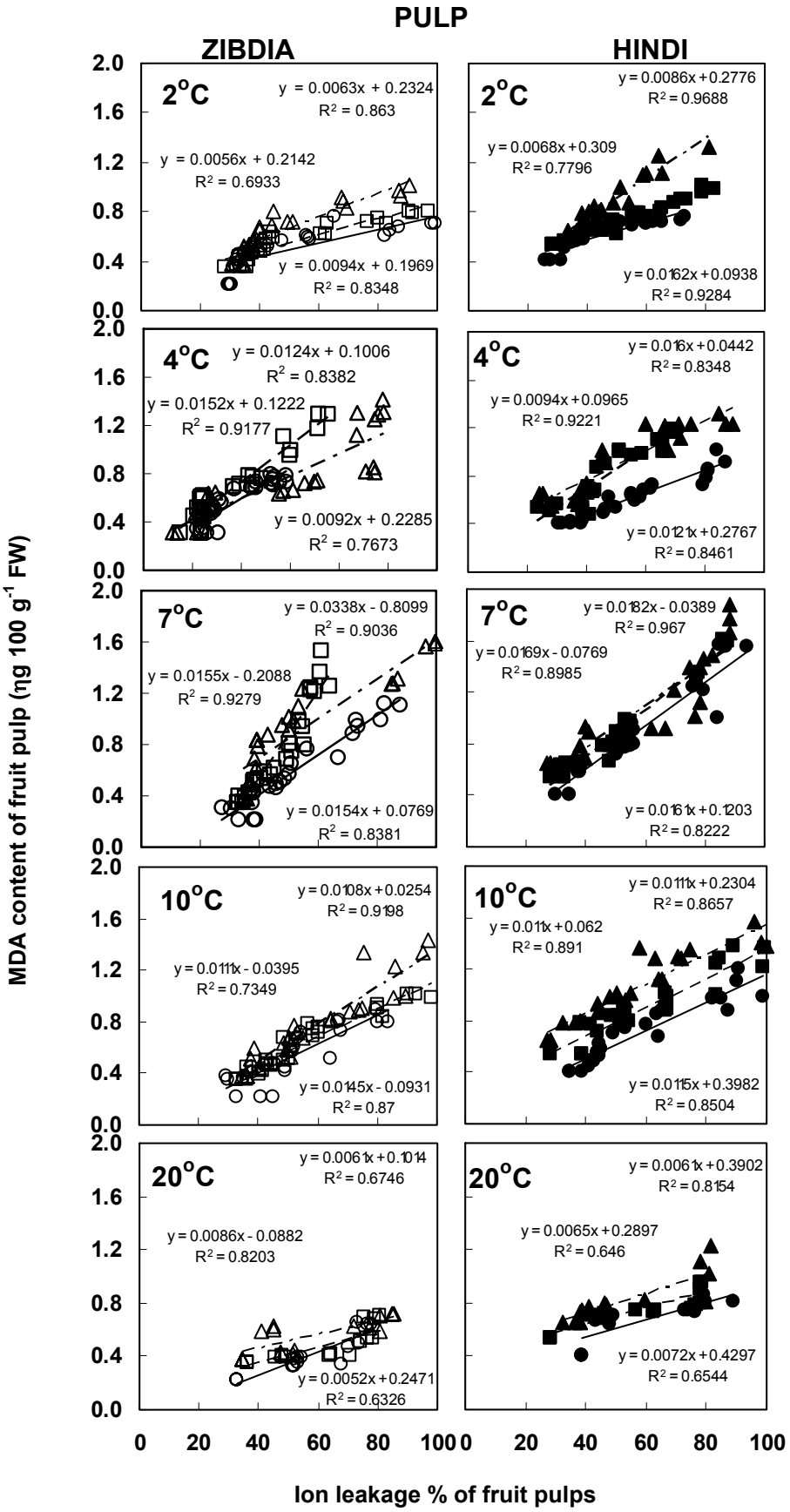

Figure 6b. The correlation between lipid peroxidation (MDA) in function α-Tocopherol content of fruit pulp of two mango varieties which stored at different storage temperatures for 35 days, (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+- 20^{0}C). Solid lines represent linear regressions.

α-Tocopherol levels also show an inverse trend to MDA levels, with the exception of the 2°C treatment, but the trend is not as clear as for ascorbic acid (Figure 11). The correlation between β-carotene content and MDA accumulation is, on the other hand, very poor (Figure 12). With the exception of the 2°C treatment, it seems therefore, that decreases in ascorbic acid and tocopherol, both of which are antioxidants that are generally active in the plant cell, are associated with increased membrane oxidation (Munné-Bosch and Alegre, 2002; Shewfelt and Del Rosario, 2000). Also, the correlations between both tocopherol and ascorbic acid levels, and MDA equivalent accumulation is similar for both Zibdia and Hindi even though their contents of these antioxidants differs (Figure 10 and 11). β-carotene, on the other hand, has antioxidant properties that are important to photosynthesizing tissues, but otherwise does not appear to an important anti-oxidant in terms of specific metabolic processes. Notably, MDA accumulation seems to be unrelated to the loss of β-carotene.

The problem with the link between the loss of active antioxidant and membrane oxidation is the response at 2°C which shows that antioxidant levels can decrease without an increase in MDA equivalents. It is possible, however, that low temperatures are protecting the membrane fatty acids from oxidation. The 4°C treatments show a greater increase in MDA with the loss of ascorbic acid than does the 2°C treatments, but it is less than the accumulation found at 7°C. This is consistent with MDA equivalent formation being inhibited by low temperatures. This inhibition could be due to various processes ranging from the initial oxidation of the fatty acid to the breakdown of the oxidized acid to the formation of the MDA equivalents.

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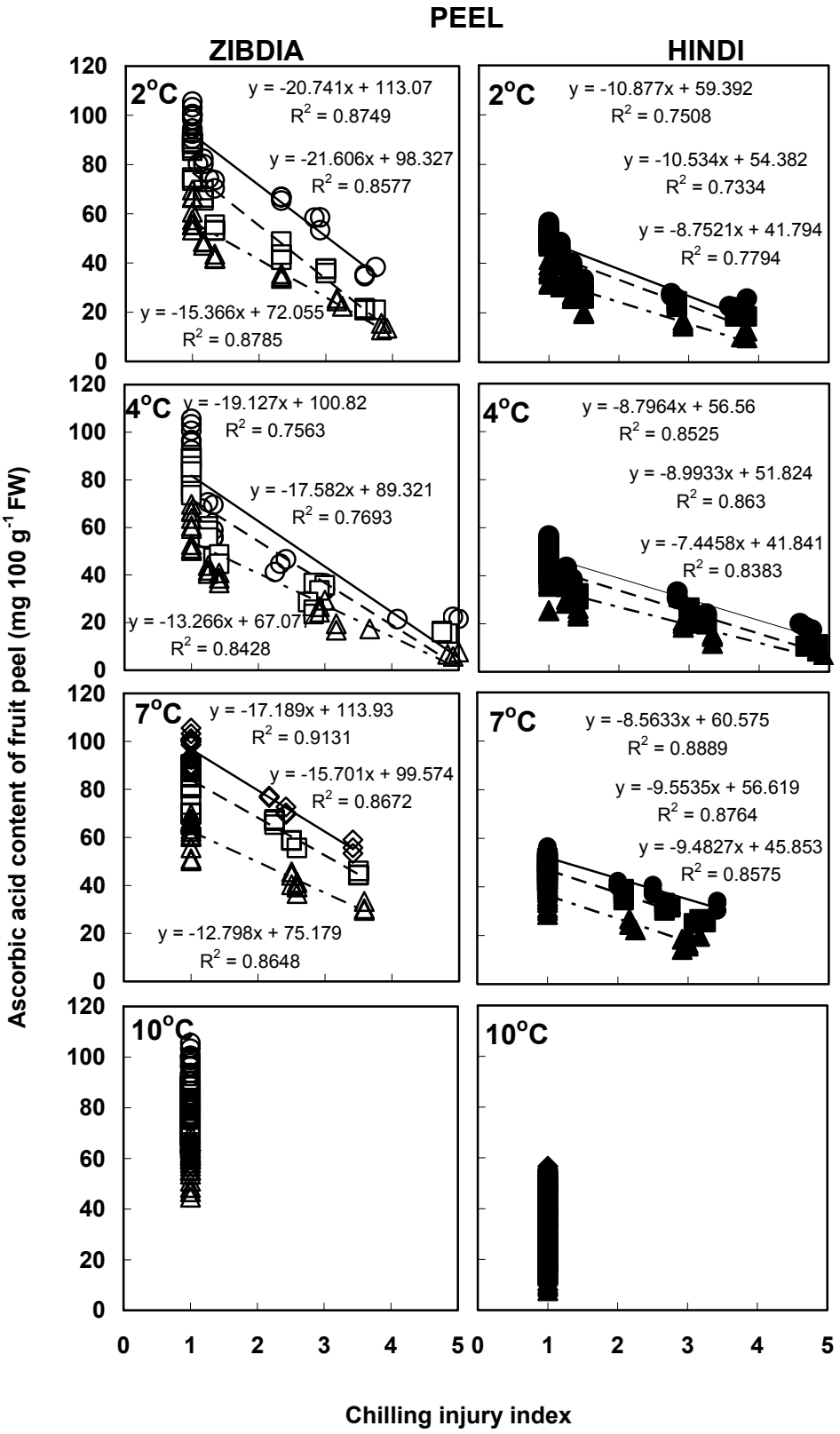

Figure 7a. The relationship of ascorbic acid content in function of chilling injury of fruits peel of two mango varieties stored at different storage temperatures for (2, 4 and 7°C) for 35 days, (Zibdia: -○- M1, -□- M2, and -△- M3) and (Hindi: - ● - M1, - ● - M2, and -▲- M3). Solid lines represent linear regressions on the data.

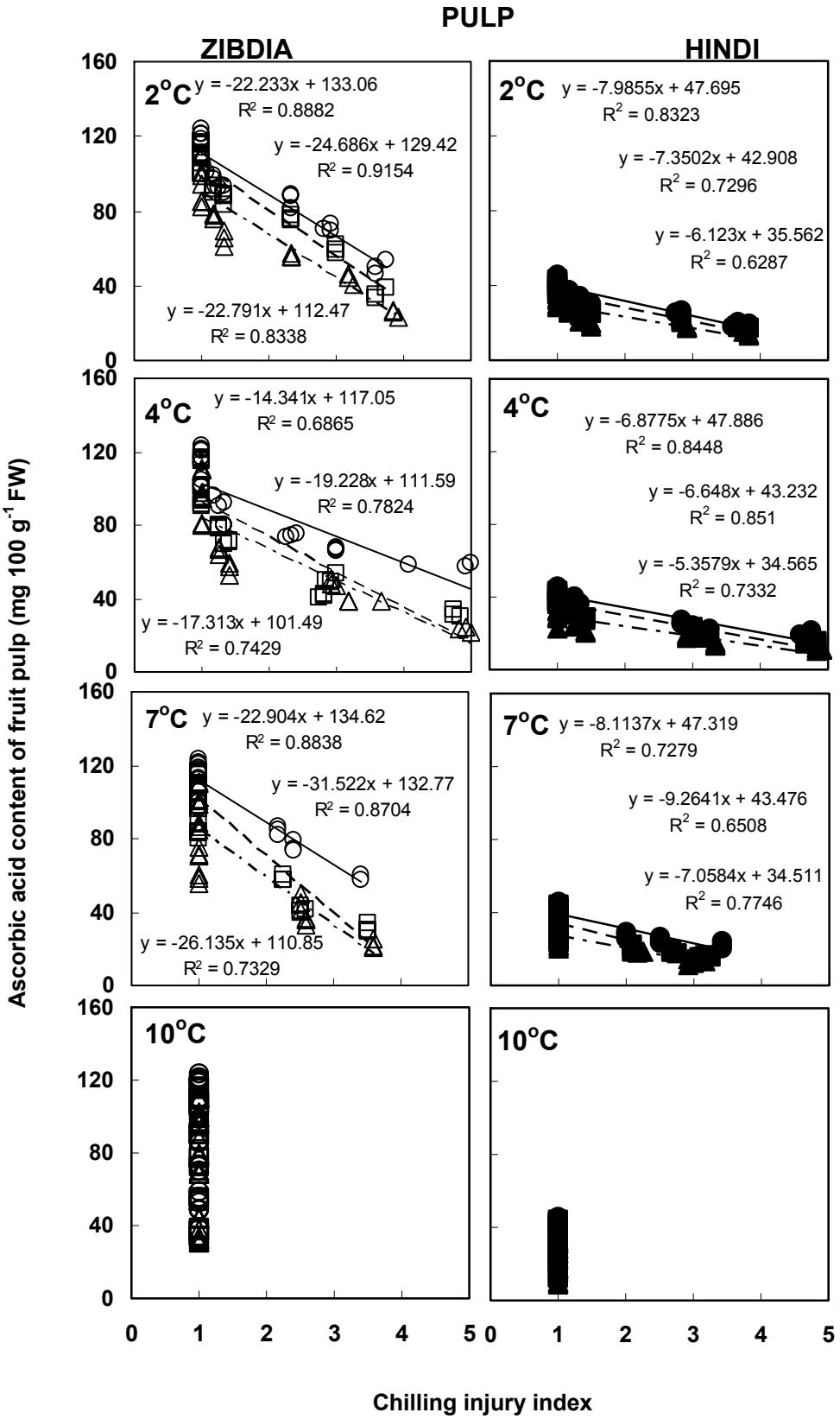

Figure 7b. The relationship of ascorbic acid content in function of chilling injury of fruit pulp of two mango stored at different storage temperatures for (2, 4 and 7°C) for 35 days, (Zibdia: -○- M1, -□- M2, and -△- M3) and (Hindi: - ● - M1, - ● - M2, and -▲- M3). Solid lines represent linear regressions on the data.

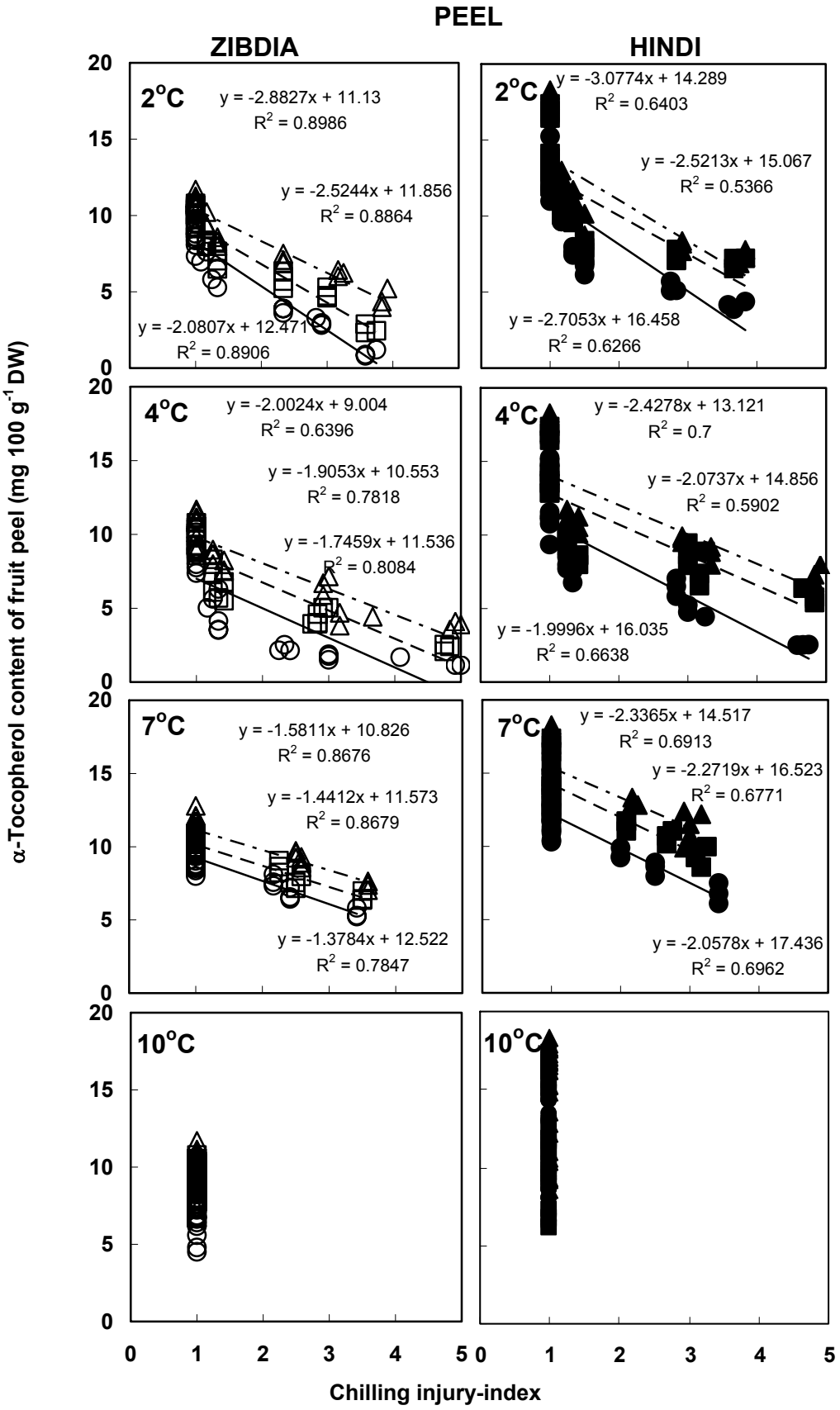

Figure 8a. The relationship of ascorbic acid content in function of chilling injury of fruit peel of two mango varieties stored at different storage temperatures (2, 4 and 7°C) for 35 days, (Zibdia: -○- M1, -□- M2, and -△- M3) and (Hindi: - ● - M1, - ● - M2, and -▲- M3). Solid lines represent linear regressions on the data.

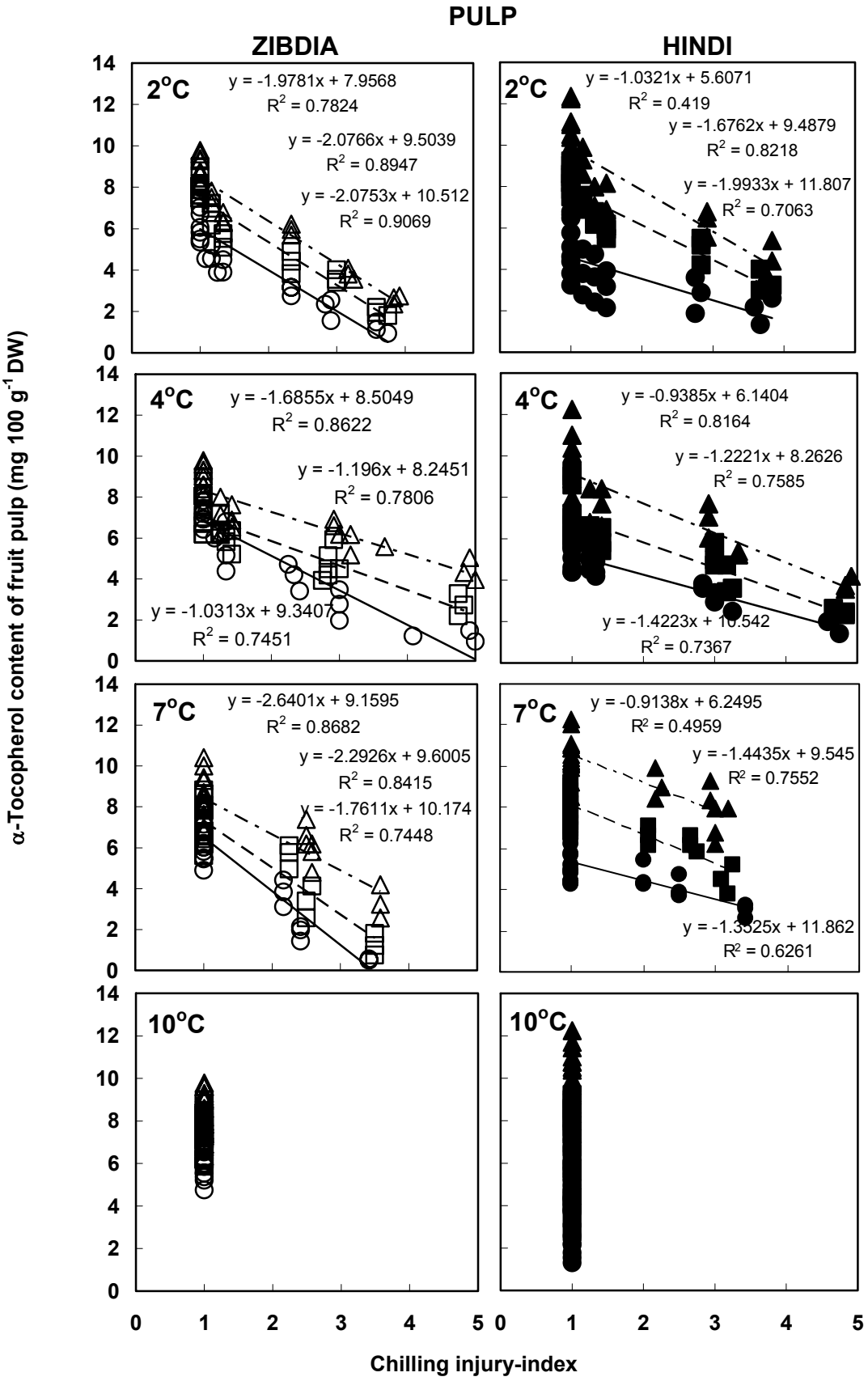

Figure 8b. The relationship of ascorbic acid content in function of chilling injury of fruit pulp of two mango varieties stored at different storage temperatures for (2, 4 and 7°C) for 35 days, (Zibdia: -○- M1, -□- M2, and -△- M3) and (Hindi: - ● - M1, - ● - M2, and -▲- M3). Solid lines represent linear regressions on the data.

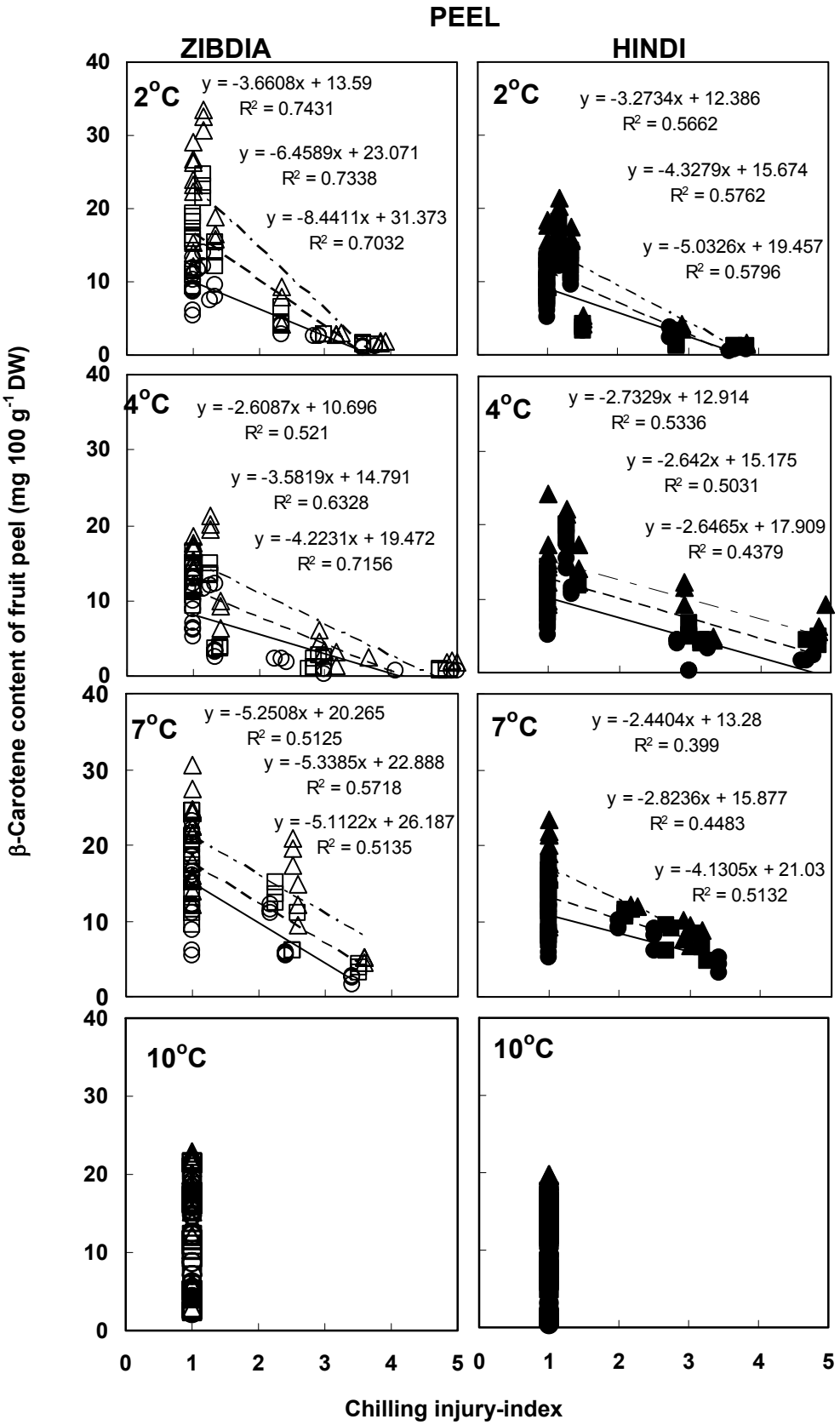

Figure 9a. presents the relationship of β-carotene content in function of chilling injury of fruit peel of two mango varieties stored at different storage temperatures (2, 4 and 7°C) for 35 days, (Zibdia: -○- M1, -□- M2, and -△- M3) and (Hindi: - ● - M1, - ● - M2, and -▲- M3). Solid lines represent linear regressions on the data.

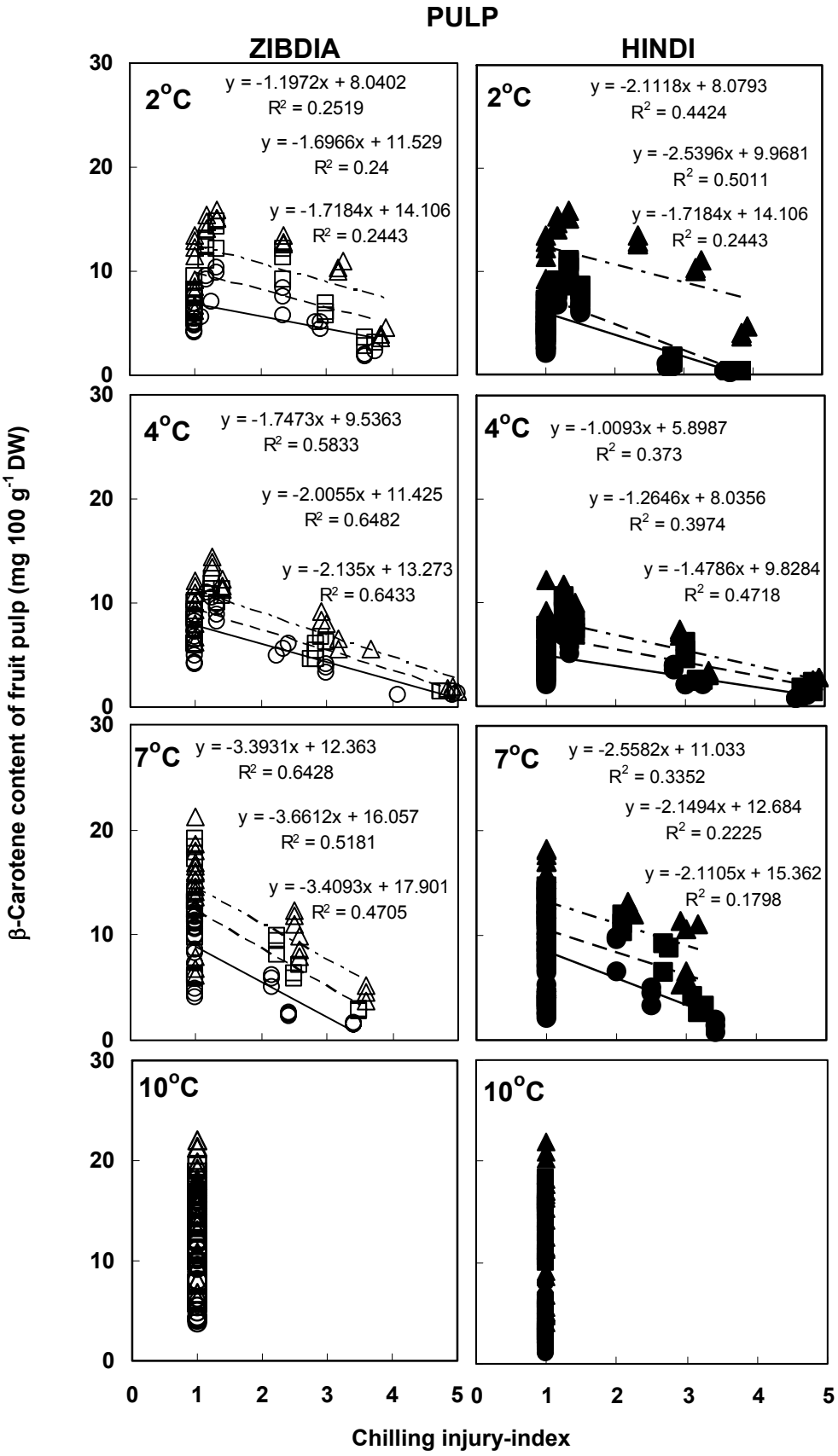

Figure 9b. presents the relationship of β-carotene content in function of chilling injury of fruit pulp of two mango varieties stored at different storage temperatures (2, 4 and 7°C) for 35 days, (Zibdia: -○- M1, -□- M2, and -△- M3) and (Hindi: - ● - M1, - ● - M2, and -▲- M3). Solid lines represent linear regressions on the data.

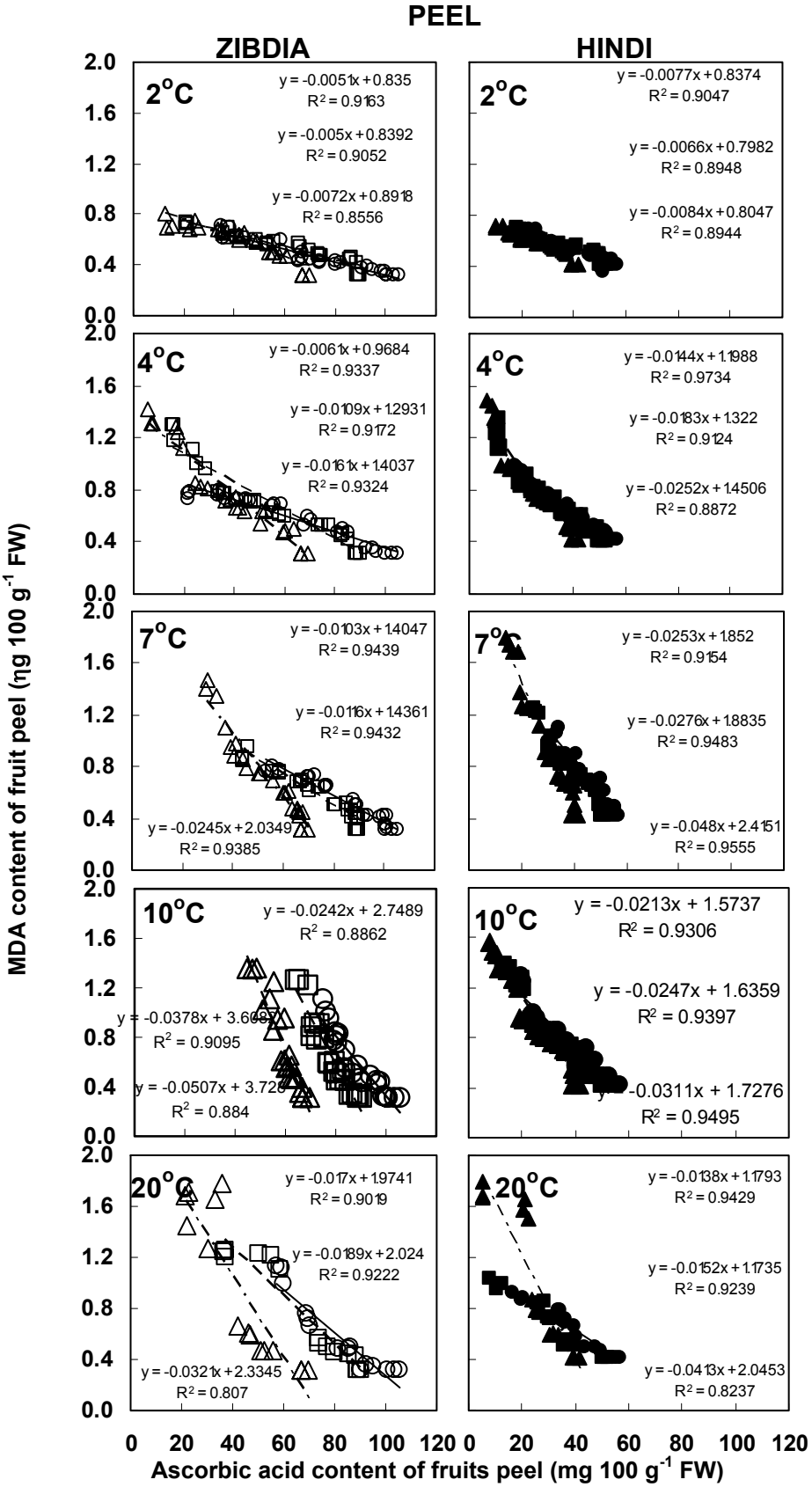

Figure 10. The relationship of malondialdehyde (MDA) content of fruits peel in function of ascorbic acid content of fruits peel of two mango varieties were harvested in three different maturity stages which stored at different storage temperatures for (2, 4, 7 and 10°C) for 35 days, (Zibdia: -○- M1, -□- M2, and -△- M3) and (Hindi: - ● - M1, - ● - M2, and -▲- M3). Solid lines represent linear regressions on the data.

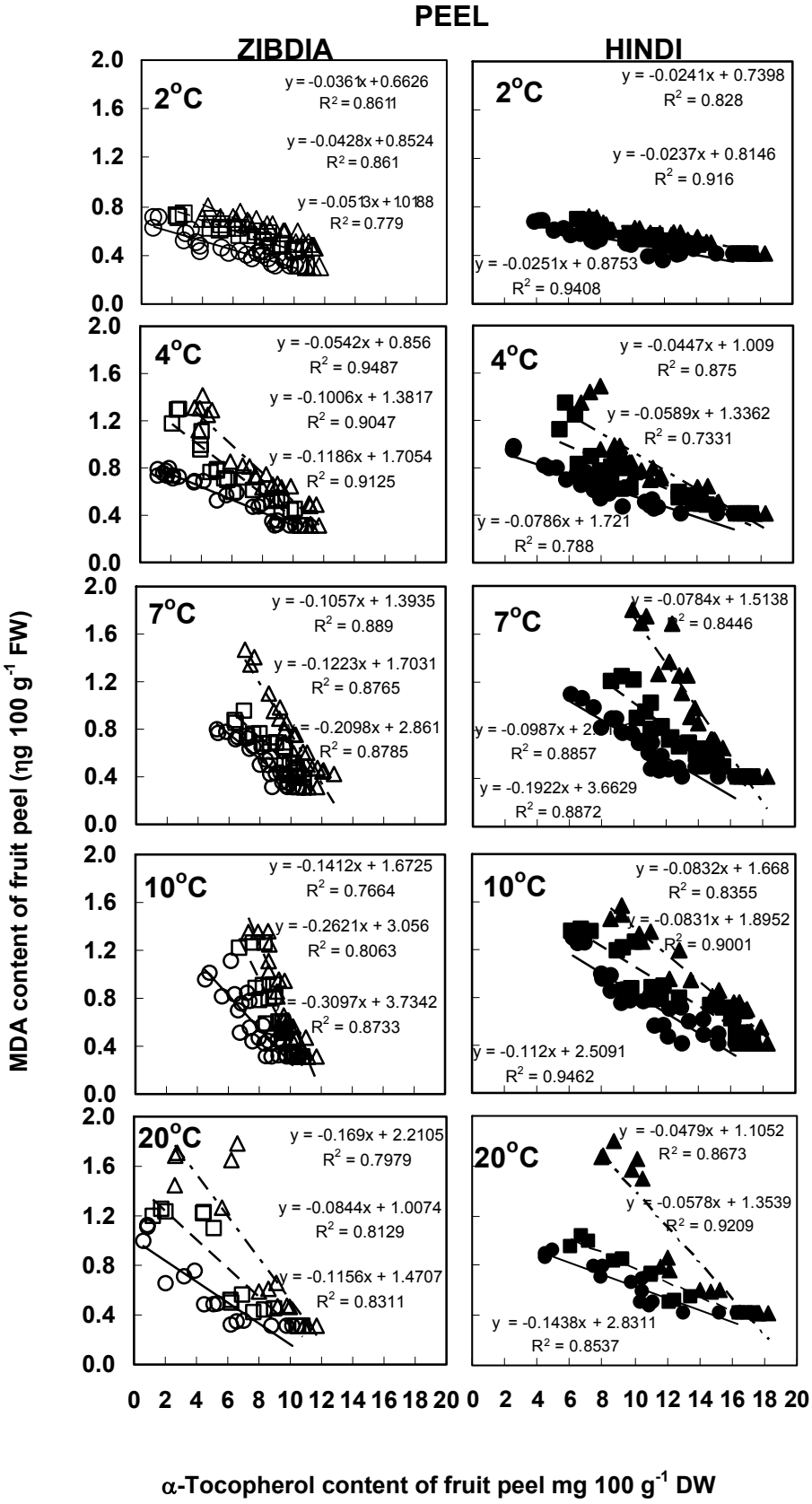

Figure 11. The correlation between lipid peroxidation (MDA) in function α-Tocopherol content of fruit peel of two mango varieties which stored at different storage temperatures for 35 days, (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+- 20^{0}C). Solid lines represent linear regressions on the data.

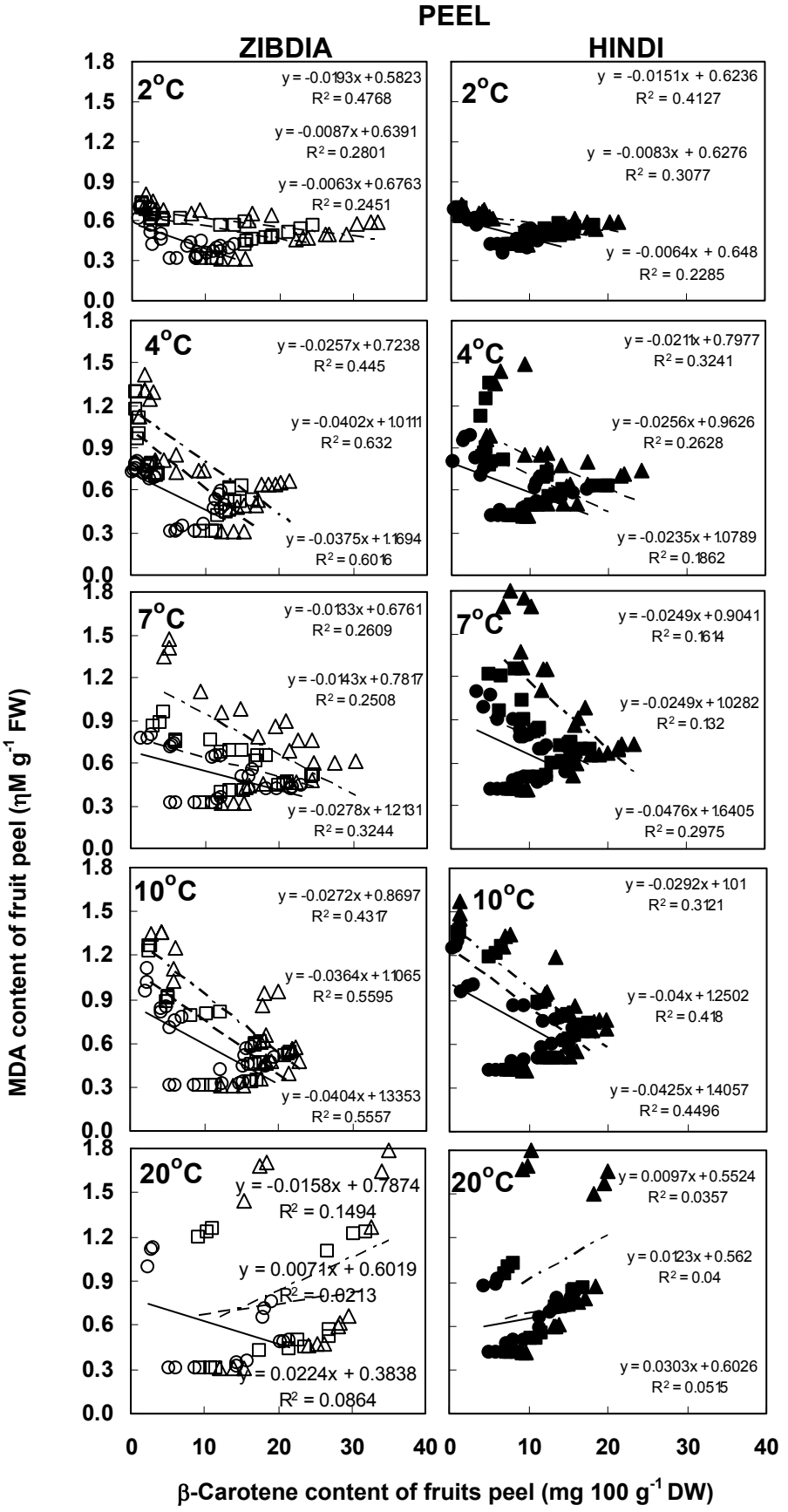

Figure 12. Presents the relationship of malondialdehyde (MDA) in function of β-Carotene of fruits peel of two mango varieties were harvest in three maturity stages which stored at different storage temperatures (2, 4, 7, 10 and 20°C) for 35 days, (Zibdia: -○- M1, -□- M2, and -△- M3) and (Hindi: - ● - M1, - ● - M2, and -▲- M3). Solid lines represent linear regressions on the data

Chapter 5

Antioxidant enzymes and oxidative stress in mangoes

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Abstract

The activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX) were measured in the peel and pulp of two mango varieties (*Mangifera indica* L. ' Zibdia' and ' Hindi Be-Sennara') during storage at 4 and 10^{o}C for periods of up to 35 days. Low initial activities of antioxidant enzymes (AE) at harvest time were observed, though this varied according to the maturity stage of the fruits at harvest. During the storage period fully mature fruits displayed the highest activities of AE compared to immature and half-mature fruits. In both varieties AE activities also increased during the first 15 days of storage, even at the lowest storage temperatures, although Zibdia showed higher activities of AE activity in this period. Thereafter, the AE activity decreased during the rest of the storage time. In conclusion, the AE activity increases up to ≈15 days of storage time after which they decreased in both the pulp and peel of both varieties and at all maturity stages. This decrease of AE leads to auto-formation of free radicals. The degree of oxidative stress pronounced by the cell is a function of the activity of free radical generation reaction on the one hand, and the activity of the free radicals scavenging system on the other hand. The imbalance between generation and scavenging free radicals are attributed to an increased lipid peroxidation, thereafter, leading to a breakdown of the thylakoid membranes. Chilling injury symptoms are clearly visible on mango fruits. The storage time and temperature are shown to be important factors in determining decreases in AE activity, which influence chilling injury presenting in mango fruits. Decreases in AE activity is varied between the selected varieties and maturity stages.

Keywords: Mango; superoxide dismutase (SOD); Catalase (CAT); glutathione reductase (GR); acerbate peroxidase (APX)

1. Introduction

Many plants and plant-products of tropical origin suffer injury and even death when exposed to low but non-freezing temperatures. Chilling sensitive fruits and vegetables will develop various fairly characteristic symptoms, or disorders, when stored for sufficient periods of time at temperatures below 15°C, depending on the species, cultivar, and tissue type. These disorders include: pitting, abnormal ripping, reduced photosynthetic capacity, necrosis and discoloration and increased disease susceptibility (Saltveit and Morris, 1990). The causes of this injury are incompletely understood but seem to involve changes in membrane organization such as decreases in the unsaturation fatty acids of membrane lipids (Nishida and Murata, 1996). Additionally, oxidative damage is associated with chilling injury, which implies that chilling treatments produce a disturbance in the cellular redox homeostasis of susceptible plant cells (Foyer and Noctor, 2000). In response to chilling treatments the antioxidant capacity of tissues is frequently observed to increase, which is consistent with the homeostasis model of Foyer and Noctor (2000). The formation of active oxygen species (AOS) may be causally related to alterations in membrane function (Foyer and Noctor, 2003), and AOS are produced in different microsomal membranes from stressed tissues (Nair et al., 2003). Therefore, the membrane and AOS models of injury seem to be connected.

The role of oxidative damage produced by AOS, and the degree to which the cell can maintain its redox balance (Noctor and Foyer, 2003) are important questions in relation to improved understanding of the processes underlying the phenomenon of chilling injury. Consistent with the idea of a shift in the oxidative balance of the cell, it is important to note that AOS are produced normally during cell metabolism, by enzymic and non-enzymic means; for example, cell membrane lipids are about 0.6 – 1.7% peroxidised in healthy tissues (Griffiths et al., 2000).

Chilling stress, along with many other stresses, shifts the AOS balance of the cell and, increases the levels of AOS (Bowler et al., 1992; Foyer and Harbinson, 1994; Hodges, 2003) and thus the level of oxidative injury experienced the cell. A range of AOS are produced by metabolic and non-metabolic processes within the cell (Evans et al., 1999; Shigeoka et al., 2002). In the absence of light the predominant primary AOS will be superoxide and hydrogen peroxide. Even in darkness the mitochondrial electron transport system continues to be a source of $O_2^{\bullet-}$radicals (Bowler et al., 1992), and $O_2^{\bullet-}$ is produced by the reduction of O_2 by NADH oxidase in the plasmalemma. From this peroxide can be formed by superoxide dismutases (Shigeoka et al., 2002), and the peroxide so formed is destroyed by various mechanisms (Shigeoka et al., 2002), which is caused many biochemical and molecular changes in cell membrane results in cell death (Rubinstein, 2000). Though superoxide and peroxide are reactive, they are much

less aggressive than the hydroxyl radical, whose reaction rate constants are close to diffusion limitation. Though the hydroxyl radical is not known to be produced directly from a metabolic process, it can be formed via the Haber-Weiss reaction between superoxide and peroxides in the presence of certain metal ions (e.g. Fe^{2+}). Once superoxide is formed, peroxide and hydroxyl radicals will form as a result of SOD activity and the Haber-Weiss reaction.

Various cell components are vulnerable to oxidation by various AOS. The unsaturated centres of the acyl chains of membrane lipids are particularly vulnerable to peroxidative chain reactions begun by an initial free-radical attack (Rubinstein, 2000). Membrane lipid peroxidation reactions make the membrane less impermeable, more vulnerable to physical damage, and more likely to phase separate at low temperatures. In addition to attacking membrane lipids, other cell components, such as lipids, proteins, carbohydrate and, nucleic acid are subject to attack result in, postharvest disorders such as superficial scald, browning and loosing of pigments, as reviewed by (Hodges et al., 2004). So, in general, the electron abstraction reactions produced by free radicals can be characterized as being indiscriminate and very harmful to living organisms (Abassi et al., 1998) Superoxide is, however, an notable exception to this rule; though it is a free radical it is not so aggressive as radicals such as the hydroxyl. This is because of its negative charge; the protonated superoxide, the hydroperoxyl radical is much reactive and as a substituent on the acyl chains of fatty acids is an active oxidant in the free-radical chain reaction. Foyer and Harbinson (1994) clearly indicated that in all the situations formation/consumption balance of AOS should be tightly controlled. Therefore, any metabolic imbalances or stress that leads to an increase in AOS which will cause damage to cell components that may be irremediable. The continuous oxidative pressure that cells are under results in their having a comprehensive protection system to guard against injury from AOS. The extent to which this protective system changes its activity in response to injury attributable to oxidative processes, or the extent to which an increase in oxidative damage correlates with decreases in the activity of certain protective mechanisms, are frequently investigated. Uncovering these correlations is valuable as *a priori* evidence of the operation of control systems working to maintain the oxidative homeostasis of the cell, as well as suggesting which processes may lead to cell injury and death. Plants have both enzymatic and non-enzymatic antioxidant systems to prevent or alleviate the damage from AOS. Many enzymes can efficiently detoxify AOS effects (e.g. lipid hydroperoxidases (Baier and Dietz, 1999) , whereas others act to detoxify various AOS before they can cause damage, for example $O^{\bullet -}_2$ is detoxified by SOD (Noctor and Foyer, 1998), and H_2O_2 is removed by catalase (CAT) and by reactions catalysed by different kinds of peroxidases e.g.

guaiacol (Lepeduš et al., 2004) and glutathione peroxidase (GPX) (Baier and Dietz, 1999). A major H_2O_2 detoxifying system in plants is the ascorbate-glutathione cycle that includes APX and GR (Asada, 1994), but this only functions in the chloroplast.

The protection systems are not perfect, however, and an increase in the production of AOS or a decrease in scavenging capacity will result in an increase in oxidative damage. Generally, however, plants show a rapid response to increased AOS produced by physical stresses (in response to, for example, changes in storage conditions e.g. temperature and time (Rogiers et al., 1998). These changes lead to an enhancement in cellular oxidants that induce an increase in the activities of natural antioxidants such as ASC, α-TOC and β-CAR, and antioxidant enzymes activity such as SOD CAT, GR, and APX (Bowler et al., 1992; Foyer and Harbinson 1994). The effects of storage conditions on the actions of the natural antioxidants were studied in detail in chapter 3. Saltveit and Morris (1990) have reported that many fruits present different sensitivities towards chilling temperatures according to their maturity stages and stage of development. The fruits stages such as ripening, aging of tissue and duration of exposure to low storage temperature have an impact on the development of chilling injury. Moreover studies comparing pre and post-climacteric Avocado, papaya and mango fruits have shown that pre-climacteric fruits are more sensitive to low storage temperatures than post-climacteric fruits (Robert, 1990).

In previous chapters damage to mango fruits produced by low temperature storage were described. These damage processes included oxidation membrane oxidation, protein oxidation and ion leakage. The changes in the anti-oxidative metabolites occurring during storage was also reported The aim of this chapter is to study the changes in the extracted activities of antioxidant enzymes such as CAT, APX, GR, and SOD during storage of two mango varieties at low temperatures., and to try and relate these changes to changes in the anti-oxidative metabolites and oxidative damage described previously. In other chapters chilling storage temperatures of2°, 4°, 7° and10°C were used. In this chapter only temperatures if 4° and 10°C were used because of the more complex assays required to measure enzyme activities; 4°C was used as it frequently produces the most damage and 10°C as it produces less, or even no, stress, depending on the property or process being measured.

2. Material and methods

2.1. Fruits harvesting and storage condition:

Fruits harvest and transport procedures and schedules are as described in chapter 3

2.2. Storage setup

Fruits were divided into two batches; each one was composed of 360 Hindi and 360 Zibdia (120 fruits at each maturity stage in three replicates). These batches were stored at 4 and 10^{o}C, and ten fruits were taken from each batch per maturity stage every 5 days (up to 35 days). From these fruits, samples of peel and pulp were taken and stored at -80^{o}C prior to analysis of ascorbic acid, SOD, CAT, GR and APX.

2.3. Antioxidant enzymes extraction and assay:

The antioxidant enzymes were extracted using to the method of (Kingston-Smith et al., 1999). Frozen mango samples (5 g) stored at – 80^{o}C were crushed into fine powder with a frozen buffer in a ball mill and the pestle under liquid nitrogen. The soluble proteins were extracted by homogenizing the powder in 5 ml of buffer consisting of 0.1 M sodium phosphate (pH 7.5), 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM dithiothreitol (DTT), 5 mM ascorbate, 10% (v/v) glycerol, 0.1% (v/v) Triton X-100, 1 mM phenylmethylsulfonylfluoride (PMSF), 1 mM *p*-aminobenzamidine, and 10 μM leupeptin. The homogenate was centrifuged at 8000g for 5 min at 4^{o}C and the supernatant was three times as replicates used for the following enzyme assays.

SOD (EC 1.15.1.1.) activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue terazolium (NBT) based on the method described by (Giannopolitis and Ries, 1977). Three ml of the reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 μl NBT, 2 μl riboflavin, 0.1 mM EDTA, and 100 μl of extract. The reaction mixtures were illuminated for 15 min using a fluorescent light. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of the NBT monitored at 560 nm.

CAT (EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 (extinction coefficient 39.4 mM^{-1} cm^{-1}) at 240 nm for 3 min (Aebi, 1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 100 μl of extract in a 3 ml volume. The units for CAT activity were μmol H_2O_2 min^{-1} g FW, but "μmol min^{-1} g FW" will be used in the text.

GR (EC 1.6.4.2) activity was assayed by measuring the oxidation of NADPH at 340 nm (extinction coefficient 6.2 mM^{-1} cm^{-1}) for 3 min in 1 ml assay mixture containing 50 mM potassium phosphate buffer (pH 7.8), 2 mM Na_2-NADPH, 50 mM GSSG and 200 μl of extract The reaction was started by adding NADPH. Corrections were made for the background of absorbance at 340 nm, without

NADPH (Schaedle and Bassham, 1977). The units for GR activity were μmol NADPH min^{-1} g FW, but "μmol min^{-1} g FW" will be used in the text.

APX (EC 1.11.1.11) activity was measured by following the decrease in the absorbance at 290nm (extinction coefficient 8.2 mM^{-1} cm^{-1}) for 1 min in 1 ml of reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 200 μl of extract. The reaction was started by adding enzyme extracts. Correction was made for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 (Nakano and Asada, 1981). The units for APX activity were μmol ascorbate min^{-1} g FW, but "μmol min^{-1} g FW" will be used in the text.

2.4 Ascorbic acid, Malondialdehyde equivalents and protein carbonyl content.

These were measured as described in chapter 4; the amounts of thiobarbituric acid reactive substances (TBARS) are expressed as malondialdehyde (MDA) equivalents.

2.5. Statistical analysis

The data were subjected to analysis of variances (ANOVA) with four factors for temperature, varieties, harvest maturity stage and storage time (days) Means were compared using the least significantly differences (L.S.D.) at p=0.05. The comparison of difference means undertaken using the least significant differences were tested at 5% probability. Linear regression analysis and ANOVA were analyzed at the 5% probability level. The statistical software package Genstat 8 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) was used

3. Results

3.1. SOD activity

Figure 1 shows the variation of the superoxide dismutase (SOD) activity (SOD activity: unit g^{-1} FW) as a function of storage time (days) for both mango varieties at two different storage temperatures (4 and 10^{o}C) in the peel (a) and in pulp (b) parts of Zibdia and Hindi mango varieties harvested at three different maturity stages (M1, M2 and M3). Clearly, the SOD activity presents a significant interaction at $p<0.001$ when the storage factors (time (measured in days), temperatures, varieties, and maturity stages) were considered. Generally, the peel and pulp regions of both mangoes types have the same initial SOD activity at harvest time, though the amount of SOD activity found did change with harvest stage at maturity. In Zibdia the SOD activity increased from 12 to 30 units g^{-1} FW)

in both pulp and peel as maturity increased from M1 to M3, whereas in Hindi fruits the increase was smaller, extending from 20 to 28 units g^{-1} FW.

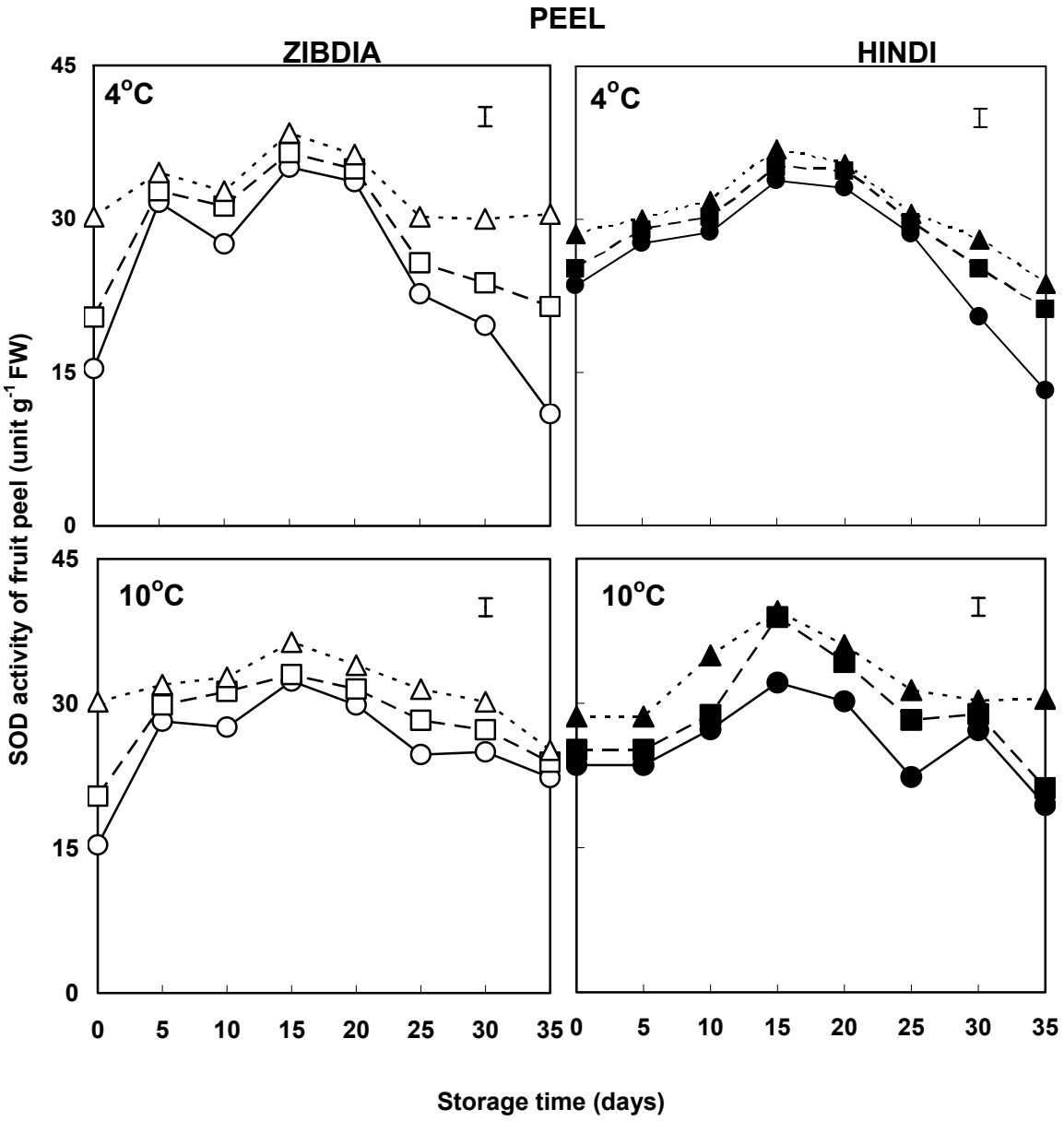

Figure1a. Superoxide dismutase (SOD) activity of peel of Zibdia and Hindi varieties harvested in different maturity stages verses storage time: immature (M1), half-mature (M2), and full mature fruits (M3). Fruits stored at 4 and 10°C for 35 days. Enzymes Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■- M2, and -▲- M3). SOD activity expressed as mean (n=3 Replicates). The vertical bars indicated to L.S.D. at *p*=0.05.

After harvest, SOD activity shows an initial period of increasing activity, reaching a maximum level at day 15 of storage for both varieties at 4 and 10°C. The value of the maximum activity of SOD increased slightly with increasing maturity stage, and was not conspicuously influenced by storage temperature, tissue type or cultivar. The maximum of SOD activity is thereafter decreases gradually till the end of storage period. The decay rates in SOD activity are more rapid at 4°C than 10°C in

both and peel and pulp of both mango varieties. Likewise the decline is greatest for M3 fruits, then M2 fruits, with M1 fruits showing the slowest decline.

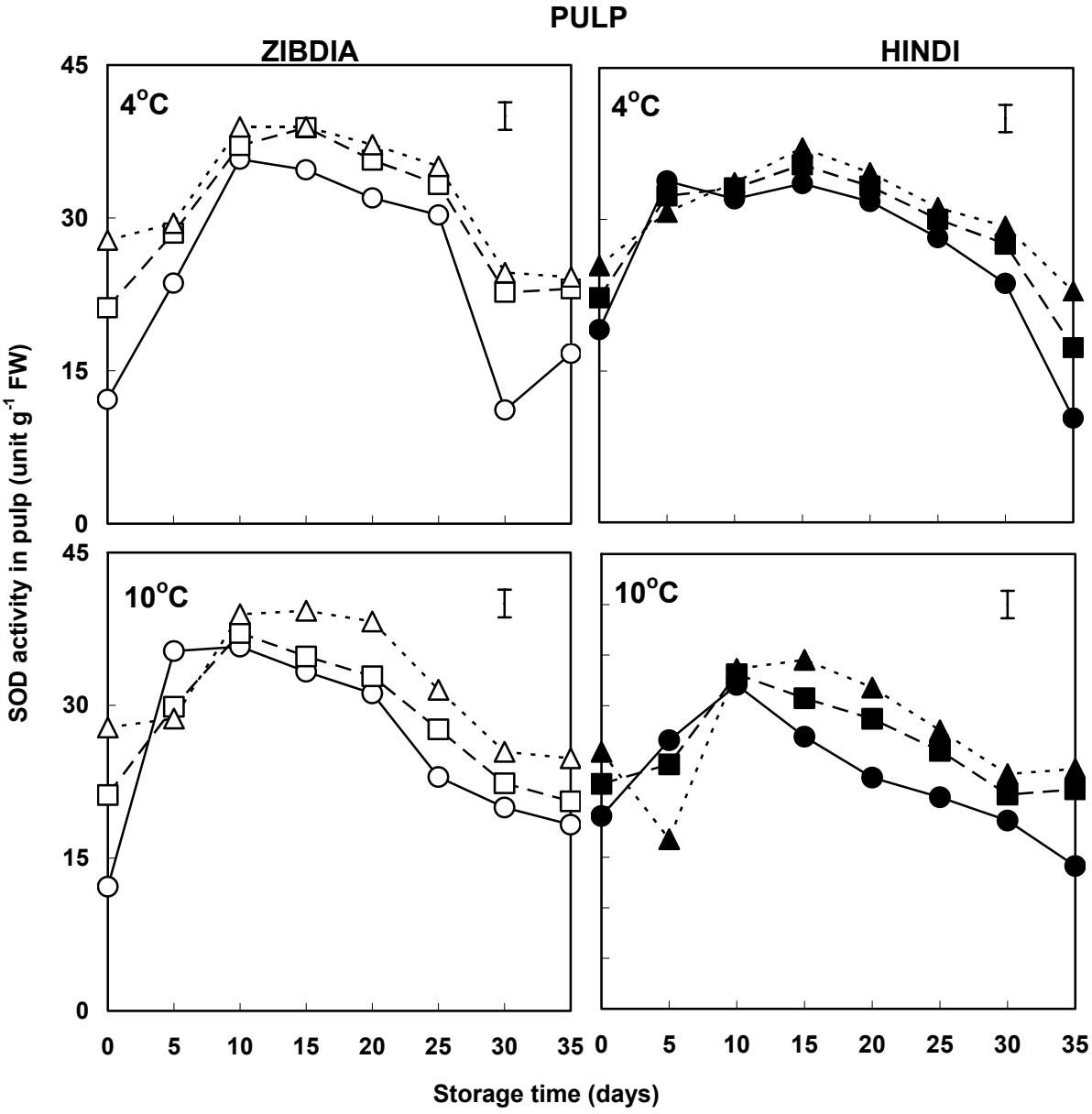

Figure 1b. Superoxide dismutase (SOD) activity of pulp of Zibdia and Hindi varieties harvested in different maturity stages verses storage time: immature (M1), half-mature (M2), and full mature fruits (M3). Fruits stored at 4 and 10°C for 35 days. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). SOD activity expressed as mean (n=3 Replicates). The vertical bars indicated to L.S.D. at *p*=0.05.

3.2. CAT activity

Figure 2 shows the variation of catalase (CAT) activity (μmol min^{-1} g FW) as a function of storage time (days) for both mango varieties at two different storage temperatures (4 and 10°C) in the peel (a) and pulp (b) of Zibdia and Hindi mango varieties harvested at three different maturity stages.

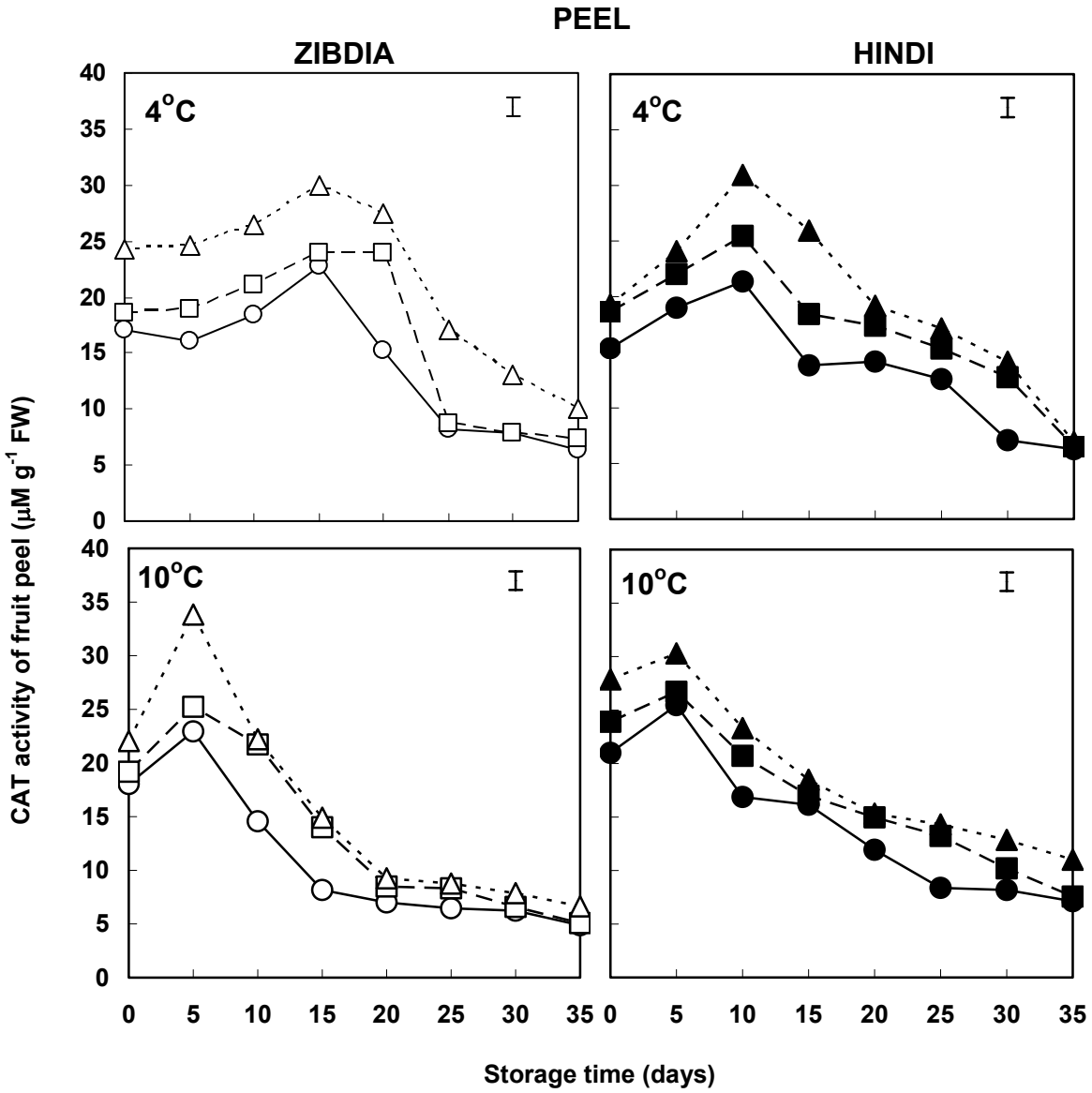

Figure 2a. Catalase (CAT) activity of peel of Zibdia and Hindi varieties harvested in different maturity stages verses storage time: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). CAT activity expressed as mean (n=3 Replicates). The vertical bars indicated to L.S.D. at *p*=0.05.

Generally, the CAT activity shows a significant interaction at p<0.001 when the storage time, temperature, varieties and maturity stages were considered. At the time of harvest the CAT activity increased with increasing maturity stage (as with SOD), but comparing varieties or tissue types, the differences were not striking. However, irrespective of maturity stage, cultivar, tissue type or storage temperature CAT activity initially increased during storage. In the case of M3 fruits of Hindi this increase were substantial: in the peel of Zibdia fruits the increase was in the range 23-53%, in the peel of Hindi it was 8-60%. Maximum CAT activities were achieved after 5 – 15 days of storage after which activity decreased to reach a minimum value after 25 days of storage or longer. CAT activity following this decrease was

either independent of maturity stage or storage temperature, or increased with increasing maturity stage. Ultimately, CAT activity was lower at the end of the storage period than it was at the beginning.

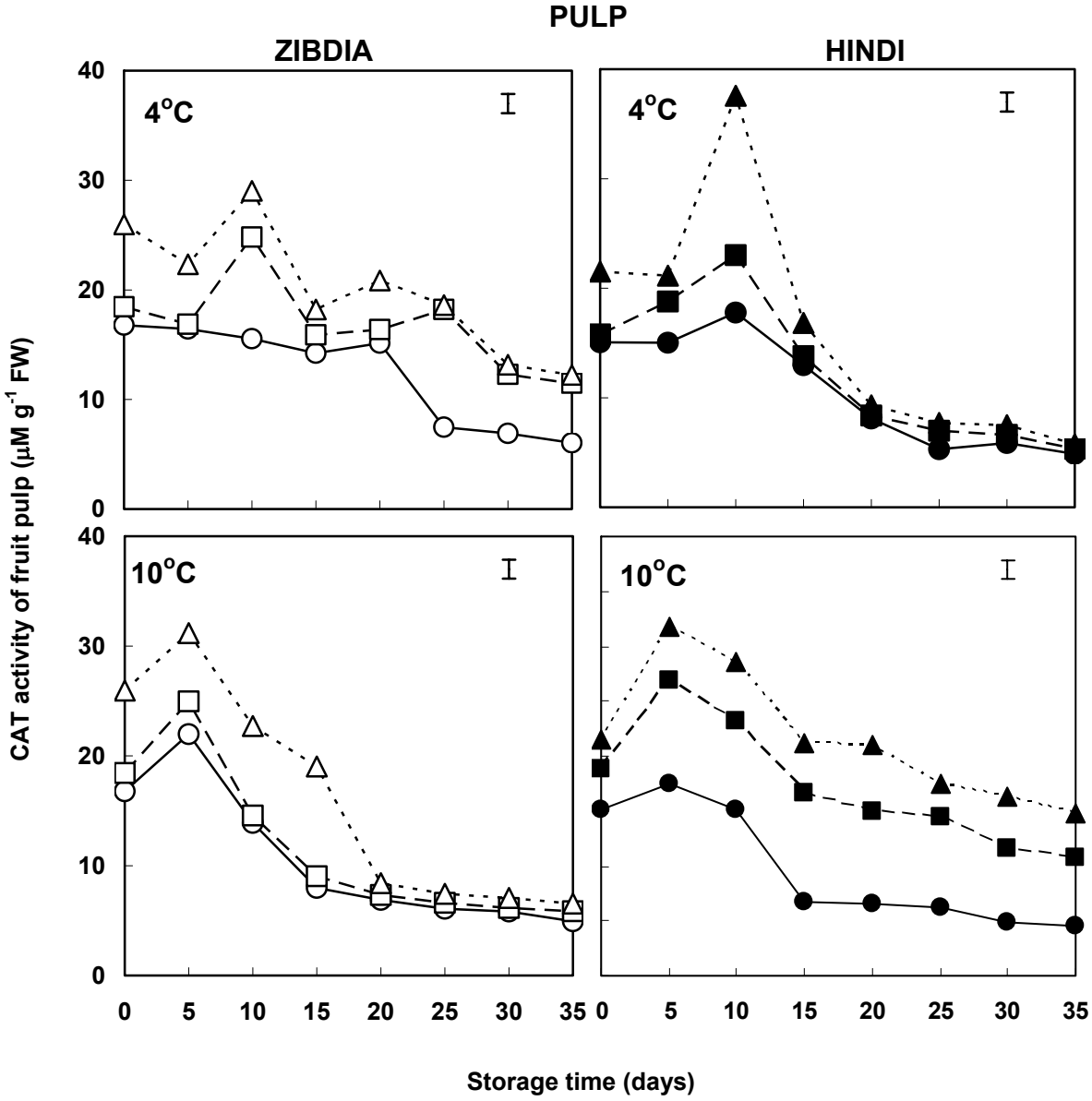

Figure 2b. Catalase (CAT) activity of pulp of Zibdia and Hindi varieties harvested in different maturity stages verses storage time: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and - ▲ - M3). CAT activity expressed as mean (n=3). The vertical bars indicated to L.S.D. at $p \leq 0.05$.

3.3. GR activity

Figure 3 shows the variation of GR activity (μmol min^{-1} g FW) as function of storage time (days), at different storage temperatures (4 and 10°C) in the peel (a) and pulp (b) for both mango varieties harvested at three different maturity stages.

In fact, the GR activity shows a significant interaction at p=0.01 when the storage time, temperature, varieties and maturity stages were considered. Generally, the peel and pulp of Zibdia fruits and the pulp of Hindi fruits had a slightly lower initial GR activity compared to the peel of Hindi fruits. The initial activities of GR was independent of maturity stage. Subsequently during storage GR activity increased reaching a maximum after 10 – 15 days of storage. This increase in activity was up to about 20-fold, and it varied depending on the tissue type, maturity stage, and temperature. In general it was greater at 4°C than at 10°C, it was greater with increased maturity stage, and for the Zibdia the increase was greater in the peel than the pulp.

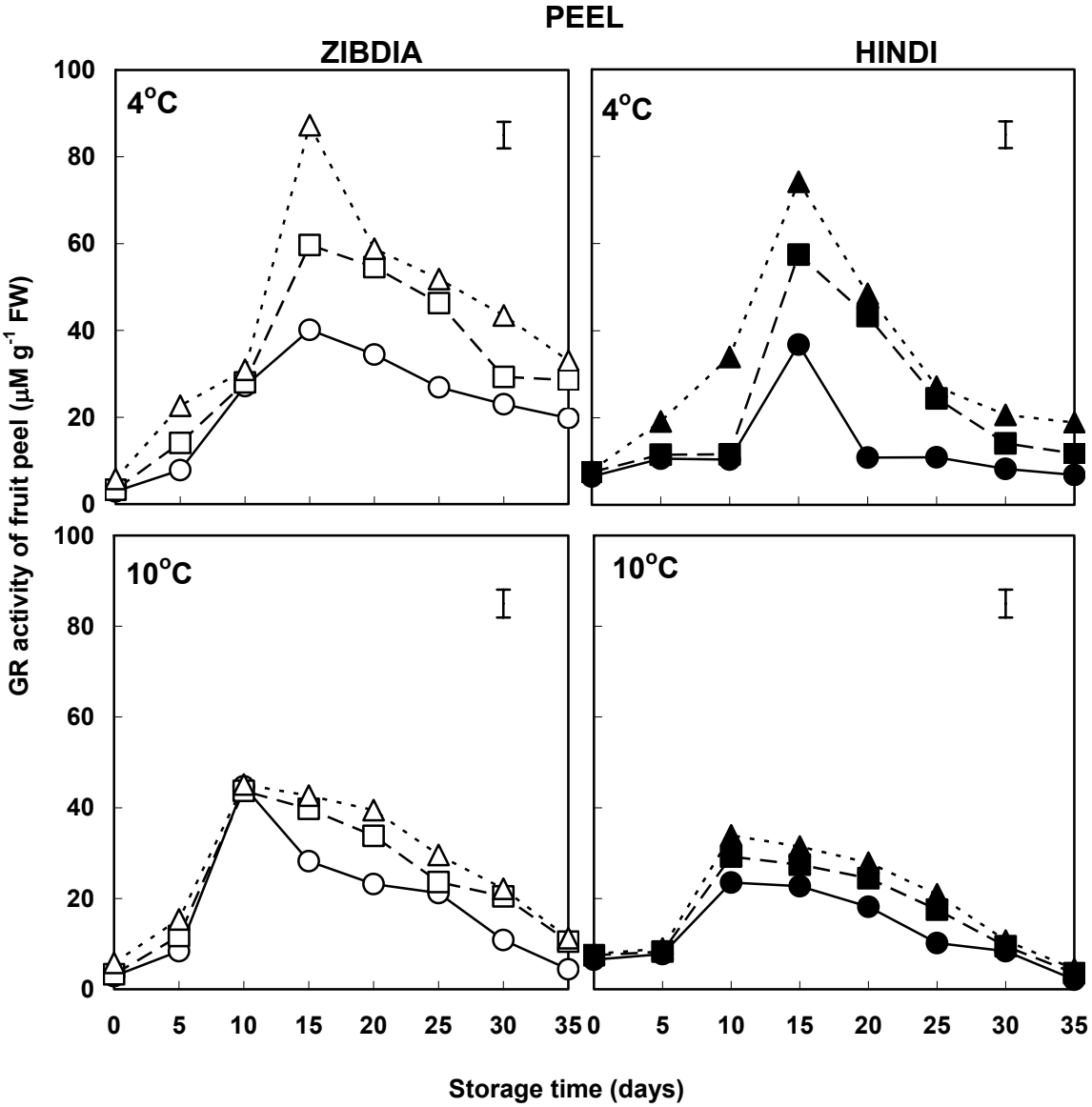

Figure 3a. Glutathione reductase (GR) activity of peel of Zibdia and Hindi varieties harvested at different maturity stages verses storage time: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■- M2, and -▲- M3). GR activity expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05.

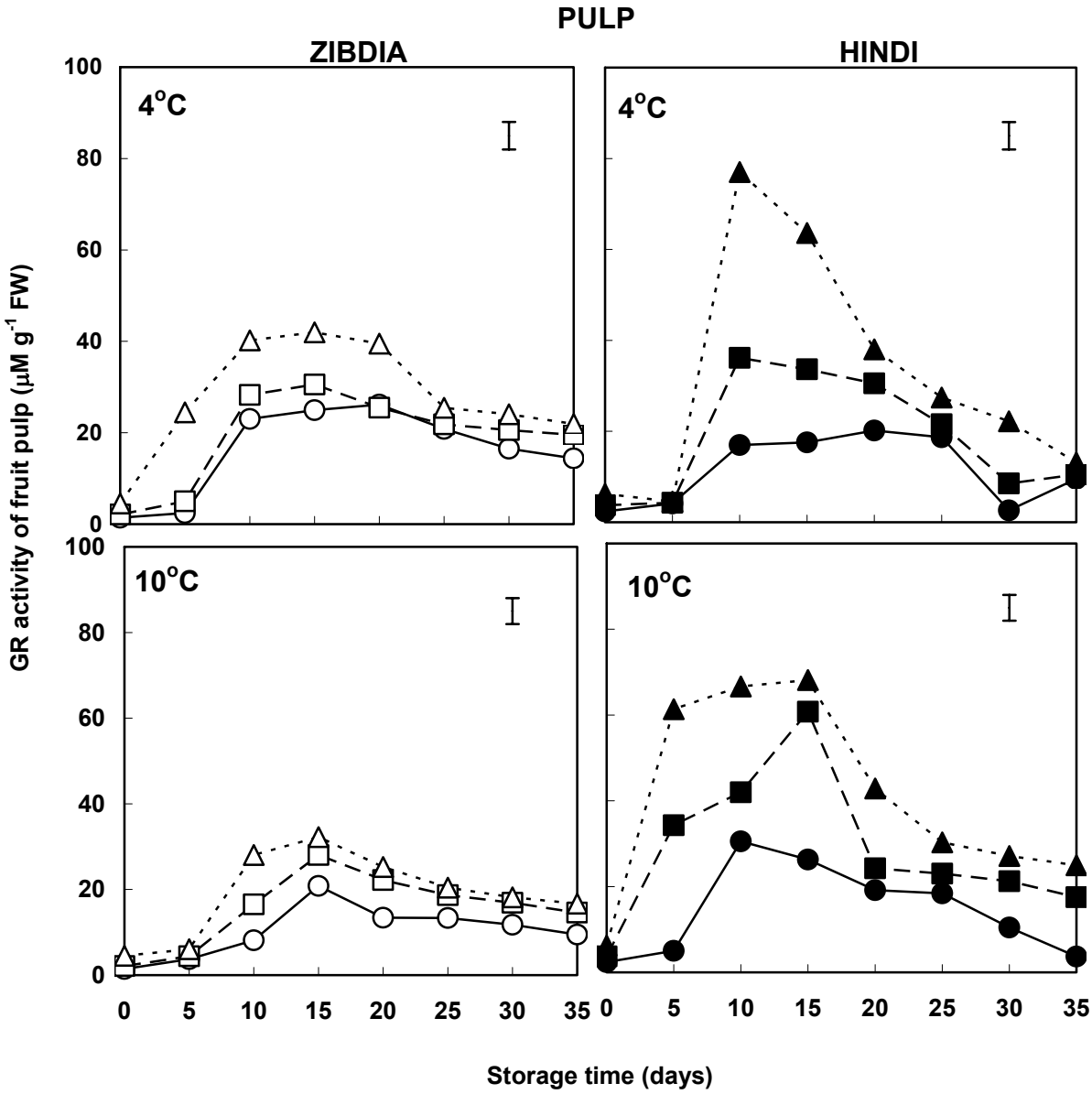

Figure 3b. Glutathione reductase (GR) activity of pulp of Zibdia and Hindi varieties harvested at different maturity stages verses storage time: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). GR activity expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05.

The maximum of GR activity is followed by a decrease in activity that extended to the end of the experiment. In most cases the activity at the end of the experiment was greater at 4°C than at 10°C, and if there was any difference between different maturity stages GR activity was greater with increasing maturity stage. At 4°C the activity at the end of the experiment was always greater than the initial activity, whereas at 10°C storage final activity was sometimes lower than initial activity.

3.4. APX activity

Figure 4 displays the variation of APX activity (μmol min^{-1} g^{-1} FW) as function of storage time (days) for both mango varieties at different storage temperatures (4 and 10°C) in the peel (a) and pulp (b). In fact, the APX activity shows a significant interaction at $p<0.001$ when the storage time, temperature, varieties and maturity stages were considered. It is apparent from Figure 7 that, both mango types have almost the initial APX activity was independent of tissue type and cultivar, but increased with increasing maturity stage.

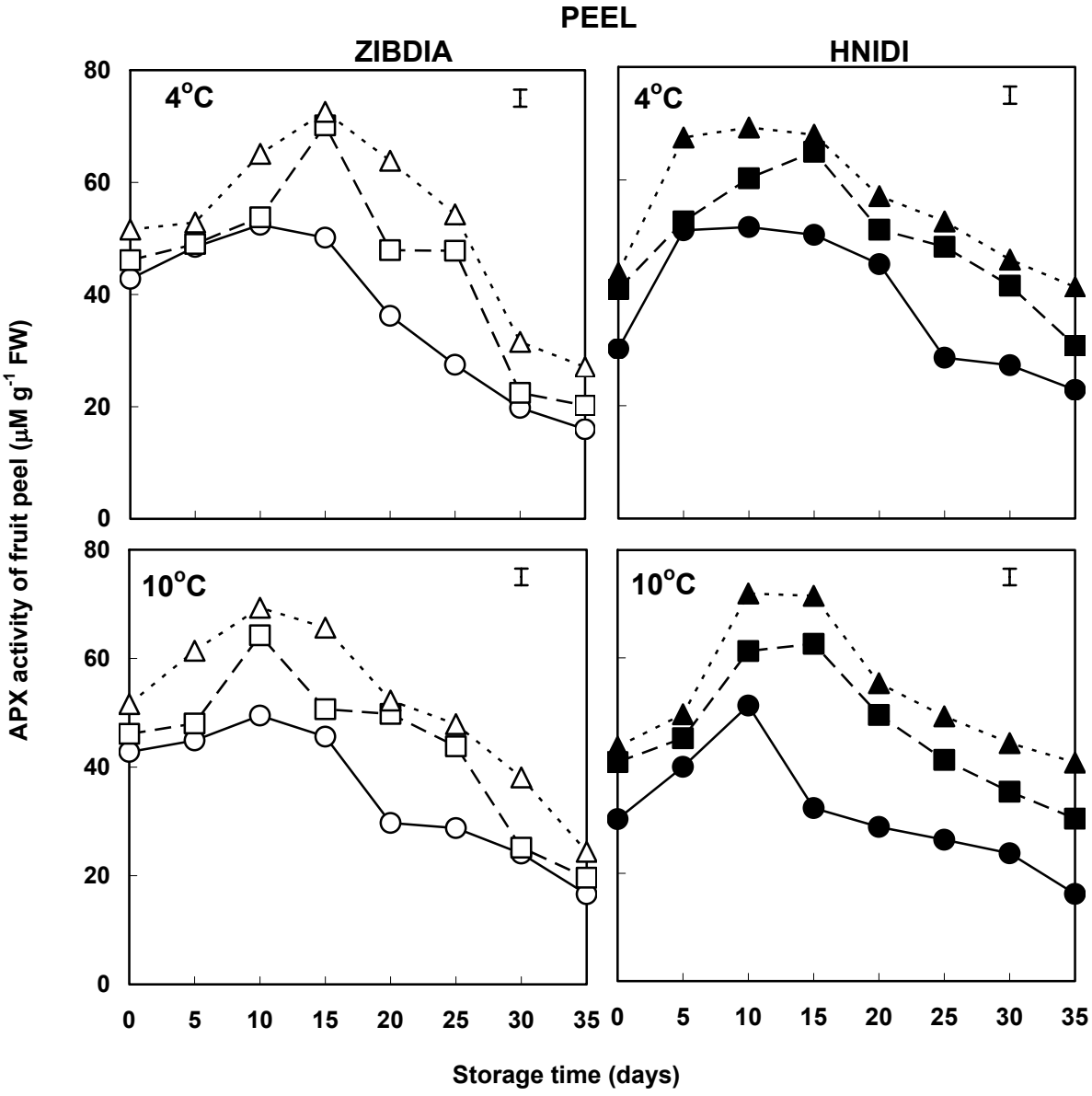

Figure 4a. Ascorbate peroxidase (APX) activity of peel of Zibdia and Hindi varieties harvested in different maturity stages verses storage time: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■- M2, and -▲- M3). APX activity expressed as mean (n=3). The vertical bars indicated to L.S.D. at $p=0.05$.

As observed with both CAT and GR, the initial activity of APX increases during storage to a maximum activity depending on the mango cultivar, the maturity stage, and the storage temperatures. The activity reaches the maximum after 5 - 15 days of storage. The maximum activity was independent of cultivar and storage temperature, but increased with increasing maturity stage. With increasing duration of storage APX activity progressively decreased, and even at the end of the storage period this decrease had not ended.

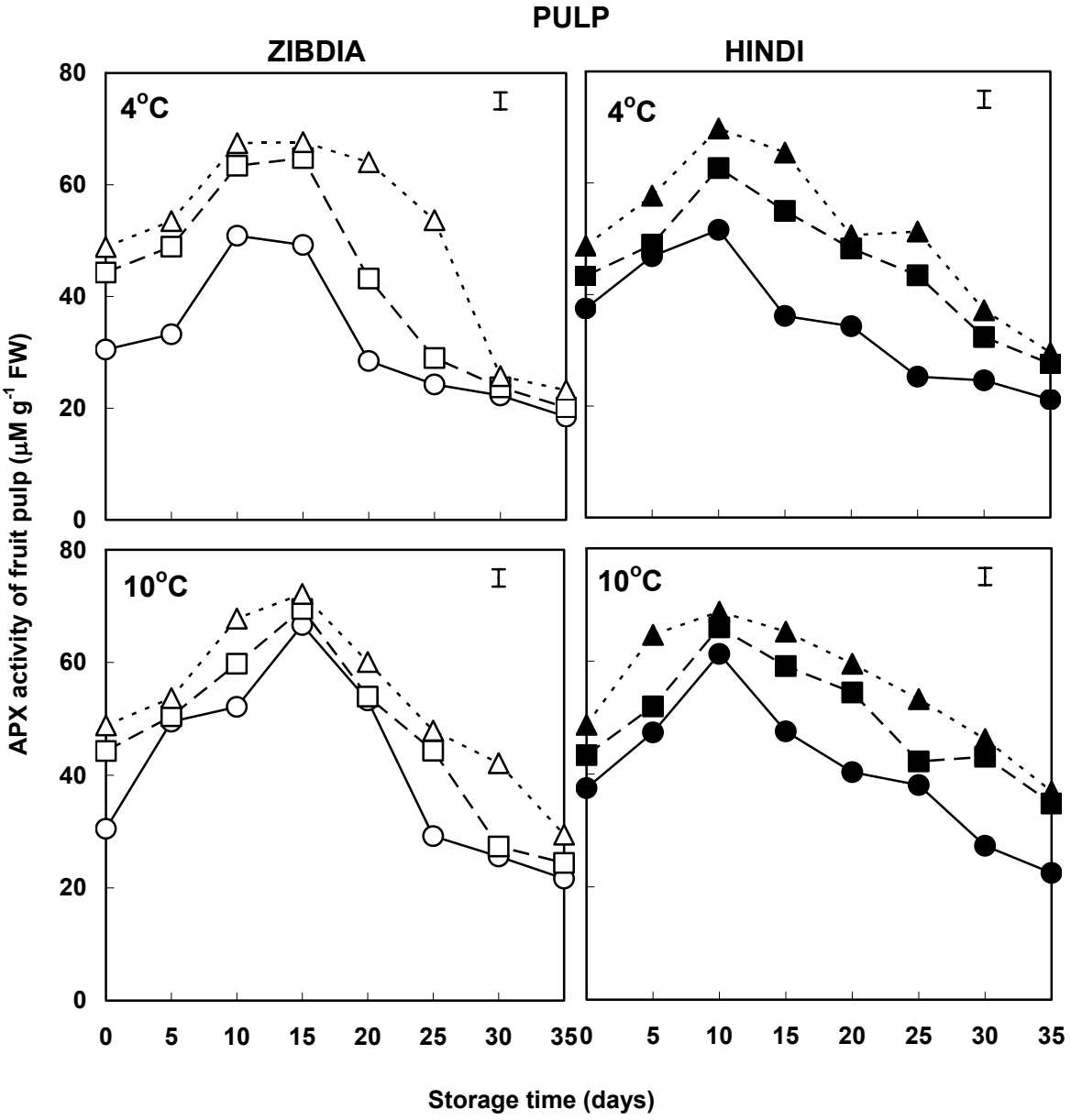

Figure 4b. Ascorbate peroxidase (APX) activity of pulp of Zibdia and Hindi varieties harvested in different maturity stages verses storage time: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■- M2, and -▲- M3). APX activity expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05.

In all cases, however, by the end of the storage treatment APX activities had decreased to below the initial activities Overall both mango varieties show a variation of APX activity dependent on fruit maturity stages, i.e. M3>M2>M1 at both storage temperatures and tissue types.

3.5 Ascorbic acid, Malondialdehyde equivalents and protein carbonyl content.

These results were described in details in chapter 4

4. Discussion

Peroxidation of cell lipid membranes with resulting in AOS formation has been described previously as one of the most damaging processes for membranes. Unsaturated fatty acids are known to easily attacked by lipoxygenase and an increased AOS production has been observed in a variety of plant tissues. The content of saturated fatty acids in membranes, lipids is strongly suggested to be directly linked to chilling tolerance in plants. However, unsaturated fatty acids are easily peroxidized by hydroxyl radicals that are formed from $O_2^{\bullet -}$ (Marangoni et al., 1996), by lipoxygenase et..

The breakdown of peroxidated lipids results in the formation of a multiplicity of low molecular weight volatile compounds (Cava et al., 2000). Because many of these compounds have low odour and taste thresholds they are responsible for flavours producing either positive or negative consumer reactions (Labuza, 1971). Marangoni et al., (1996) have reported that the extent to which the autooxidation of fatty acids and esters contributes to the formation of volatile compounds depends on both the chemical structure of the fatty acid and the process and/or the storage temperature. In principal, unsaturated fatty acids are much more sensitive than saturated ones towards autooxidation and only the former become more sensitive to oxidation under normal storage conditions. The mechanism of lipid peroxidation has been discussed in Chapter 2. Malondialdehyde (MDA) and other aldehydes constitute one of the major groups of products formed following lipid peroxidation, so determination of these compounds as thiobarbituric acid reactive substances is, therefore, a sign of the degree of lipid peroxidation. Storage of mangoes at 20^{o}C caused rapid increase in the MDA content during storage (Chapter 4), which implies membrane oxidation and breakdown. Membrane oxidation alters bilayer stability and increases the permeability of the membrane (see chapter 4). The same feature has previously been observed during the membrane breakdown which occurs during normal ripening (Hodges, 2003; Meir, 1986). More important for this study are the responses that occur when mango fruits were stored between 4 and 10^{o}C. It is clear that lipid peroxidation (MDA) increases as the storage

temperature is decreased, though in a small number of treatments (e.g. M2 and M3 Zibdia peel and M3 Hindi pulp) the greatest damage occurred at 4°C, not 2°C. These results indicate that low storage temperatures increase lipid peroxidation of cell membrane, which also correlates with decreases in ascorbic acid and tocopherol contents, and increases in electrolyte leakage (chapter 4). This is consistent with the idea that the consequences of the peroxidation process are losses in cell membrane integrity, physical structure, membrane fluidity, and increase permeability. Overall, Zibdia fruit pulp accumulates less MDA than does the pulp of Hindi, but the relationship between MDA accumulation and ascorbic acid content is similar for both fruit varieties at each storage temperature. This strengthens the proposition that oxidative damage is occurring, and that the rate of damage is inversely dependent upon antioxidant capacity as suggested by the redox homeostasis model of Foyer and Noctor (2000).

In addition to membrane oxidation, oxdatively modified forms of proteins also accumulate during storage (chapter 4). This is an oxidative stress that is initiated by and dependent upon the presence of AOS; hydroxyl radicals are thought to initiate the reaction by forming protein radicals which then react further with O_2, $O_2^{-\cdot}$, or $HO_2^{\cdot}$, or oxidation may occur by the reaction of proteins with the breakdown products of lipid peroxidation (Berlett and Stadtmann, 1997). Collectively, these active oxygen species lead to oxidation of amino acid residue side chains, formation of protein-protein cross linking, and oxidation of the protein backbone, and finally, protein fragmentation (Berlett and Stadtman, 1997). The presence of carbonyl groups on proteins has been used as a marker of AOS-mediated cell oxidation and as it is relatively easy to detect using simple protocols, so it is a relatively sensitive, reliable indicator (Levine et al., 1994). It is clear from our data that as with membrane oxidation the protein oxidation processes depend on storage temperatures (Chapter 4). The effect of decreased storage temperatures is to enhance the oxidative reactions, though in contrast to MDA formation, the greatest oxidation always occurs at 4°C, not 2°C.The relationship between protein oxidation (PCG) and AE (APX, CAT and SOD), shows that these is an increase in PCG when AE activities decreased after 15th day of storage (Figure 5, 6 and 7).
Therefore, it seem that the increases of AOS formation during the first 15 days of storage are parallel to increases of AE activities, thereafter, AE decreased up to 35 days. These relationships were different depend on variety and maturity stage;, the effects of maturity stage, storage time and temperature have been reviewed by (Hodges et al., 2004). All four enzymes, SOD, CAT, GR, and APX, show similar trends during storage.

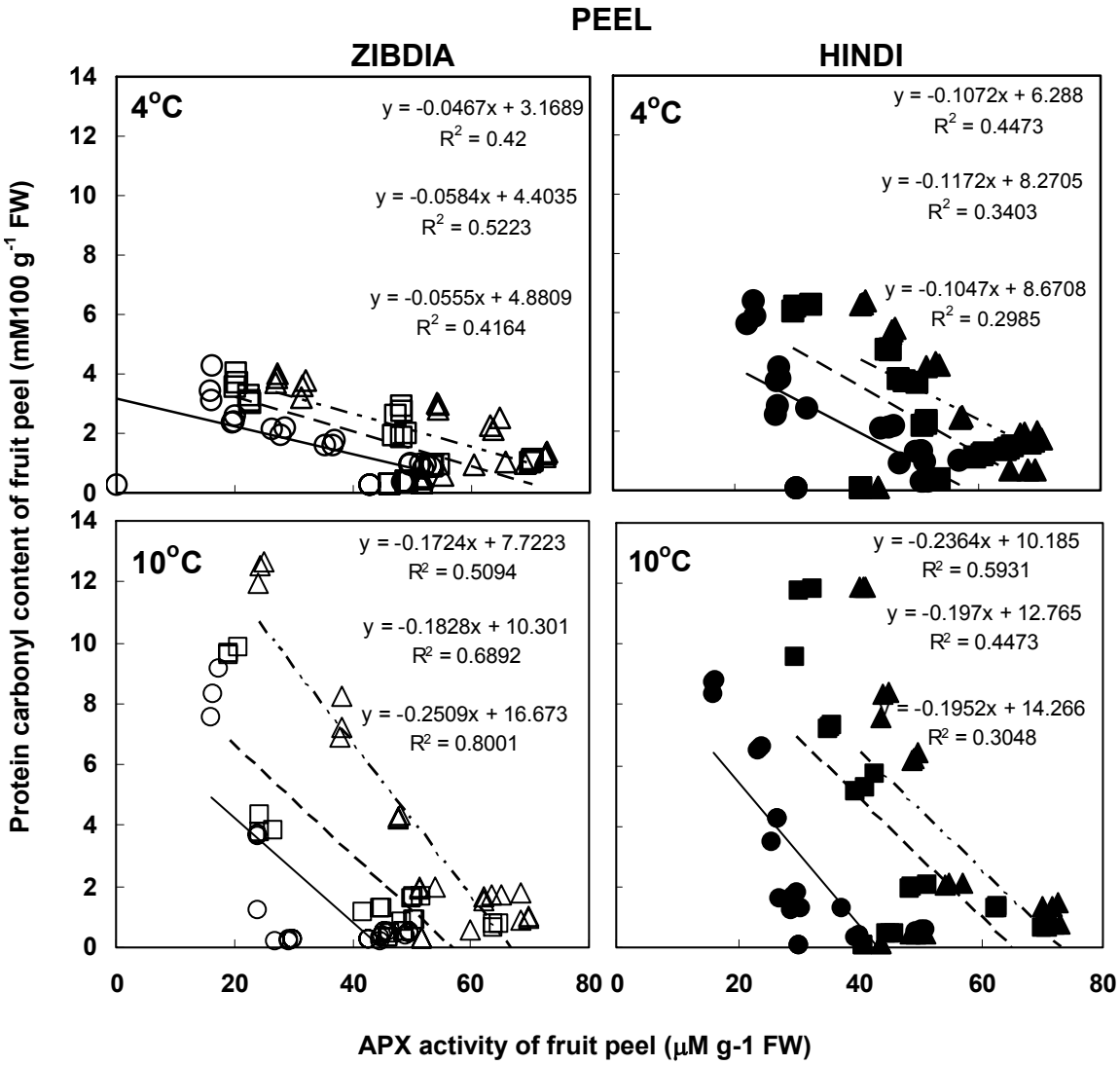

Figure 5a. The relationship protein carbonyl (PCG) content in fruits peel in function of ascorbate peroxidase activity in fruits peel of two mango varieties Zibdia and Hindi were harvested in different maturity stages were stored at 4 and 10°C for 35 days. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

They all show some degree of increase during the storage period, and subsequently a decline in activity. Where activity is dependent on maturity stage it increases with increasing maturity stage, and where activity depends upon tissue type, the fluctuations in activity are more dramatic in the peel compared to the pulp. Surprisingly, the increases in enzyme activity occur even at 4°C, and in some cases the increases are greatest at this temperature. This implies that though this is a chilling sensitive tropical fruit, it is still capable of protein biosynthesis, which implies a high level of metabolic control and coordination. Notably, β-carotene content also increases during the cold storage of mangoes (Kader, 2002) chapter 4, reaching a maximum after 15 days at 4 ° C, and 10°C. This increase also implies coordinated metabolic activity. Increases in chilling-injury index and, less clearly ion leakage, occur after 15 days of cold storage (Chapter 3), so the maximum of

enzyme activity may be determined by the onset of cell damage induced by the cold-storage.

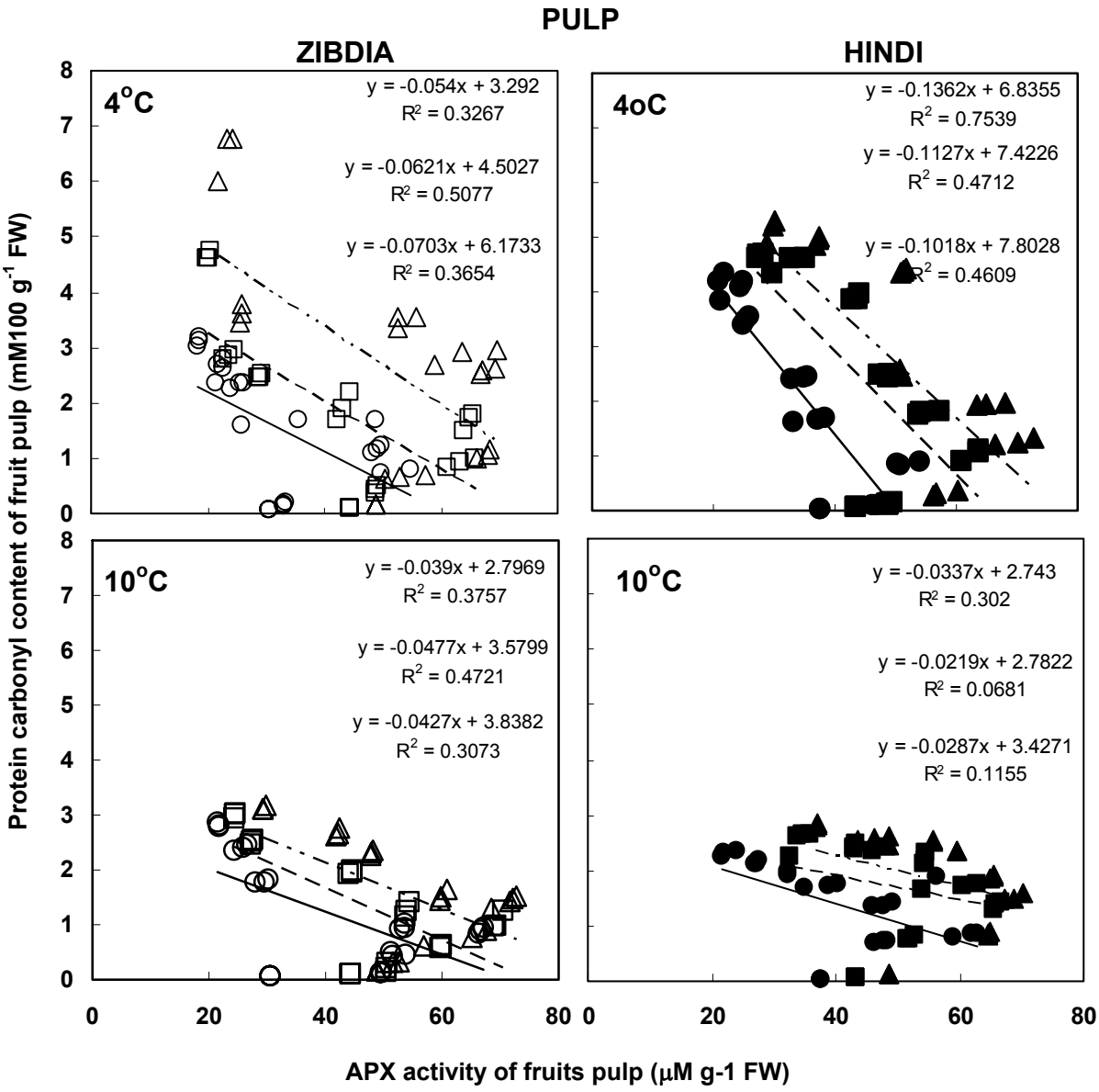

Figure 5b. The relationship protein carbonyl (PCG) content in fruits pulp in function of ascorbate peroxidase activity (APX) in fruits pulp of two mango varieties Zibdia and Hindi were harvested in different maturity stages were stored at 4 and 10°C for 35 days. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

The responses of anti-oxidant enzymes are different to those of the changes in the pool size of anti-oxidant metabolites (ascorbic acid α-tocopherol and β-carotene). Both ascorbic acid and tocopherol show continuous decreases in pool size with increasing duration of storage. Only the β-carotene pool displays the increase in size found with the enzymes.

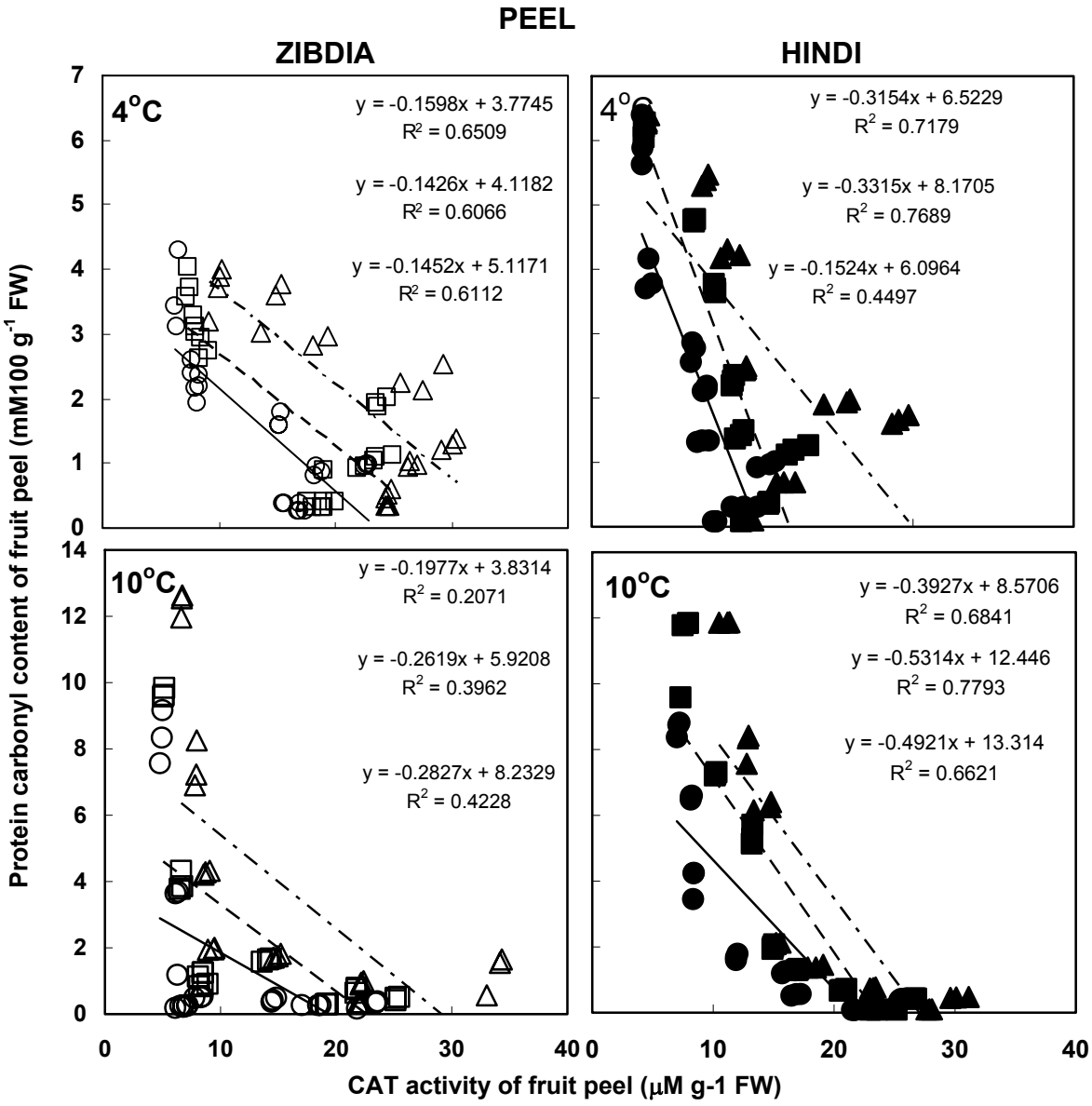

Figure 6a. presents the relationship protein carbonyl (PCG) content in fruits peel in function of catalase activity (CAT) in fruits pulp of two mango varieties Zibdia and Hindi were harvested in different maturity stages were stored at 4 and 10°C for 35 days. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

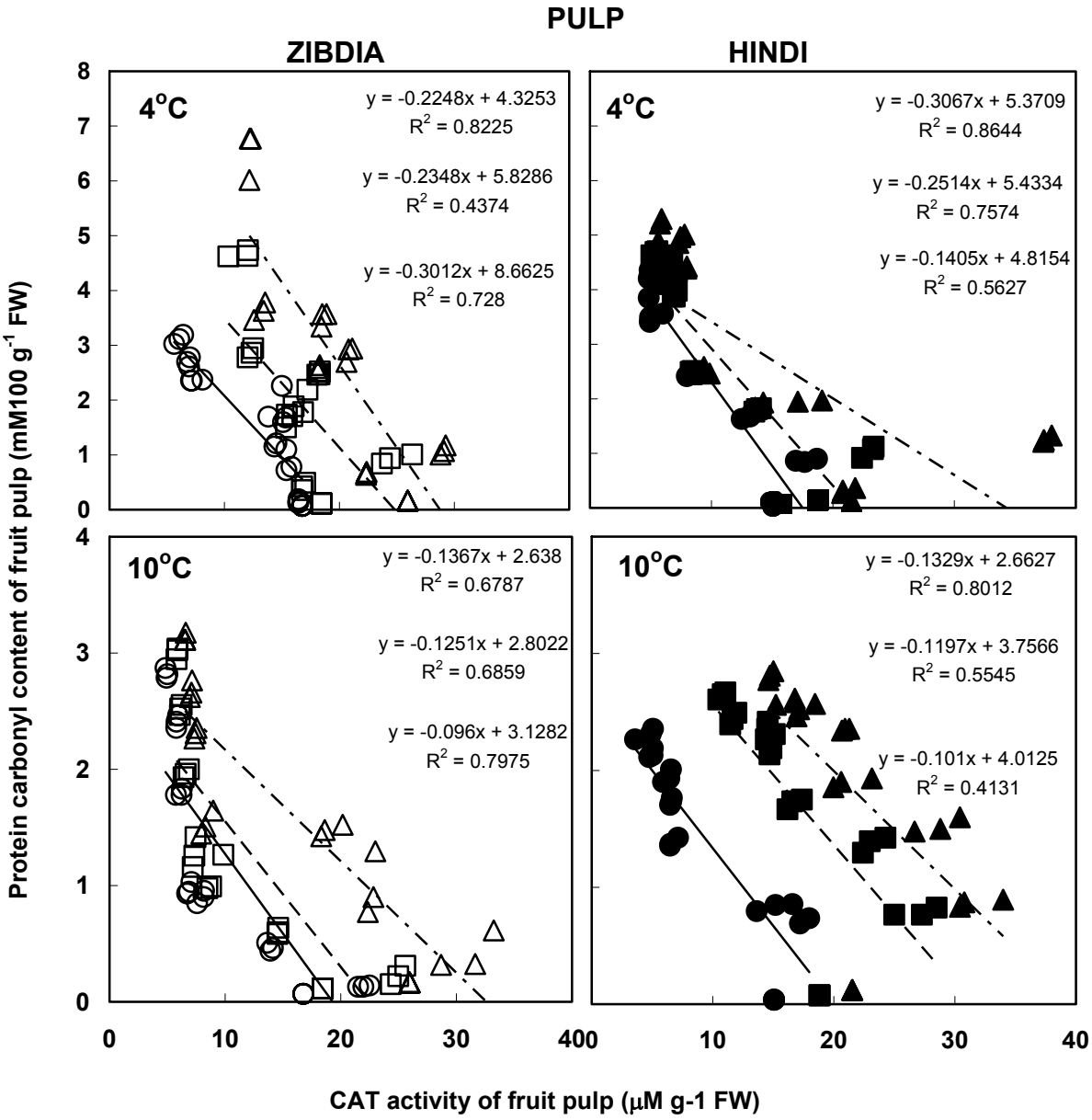

Figure 6b The relationship protein carbonyl (PCG) content in fruits pulp in function of catalase activity (CAT) in fruits pulp of two mango varieties Zibdia and Hindi were harvested in different maturity stages were stored at 4 and 10°C for 35 days. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

This is in contrast to changes in anti-oxidant pool sizes and enzyme activities found in other tissues where changes typically occur in parallel. To explore to what extent the changes in anti-oxidant enzyme activity correlates with cell injury the relationship between anti-oxidant enzyme activity and chilling injury and ascorbic acid levels was further explored (Figure 8 and 9). The production of thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde equivalents (MDA equivalents) increases during the cold storage of mangoes (Chapter 3) due to the oxidation of unsaturated acyl groups of membrane lipids.

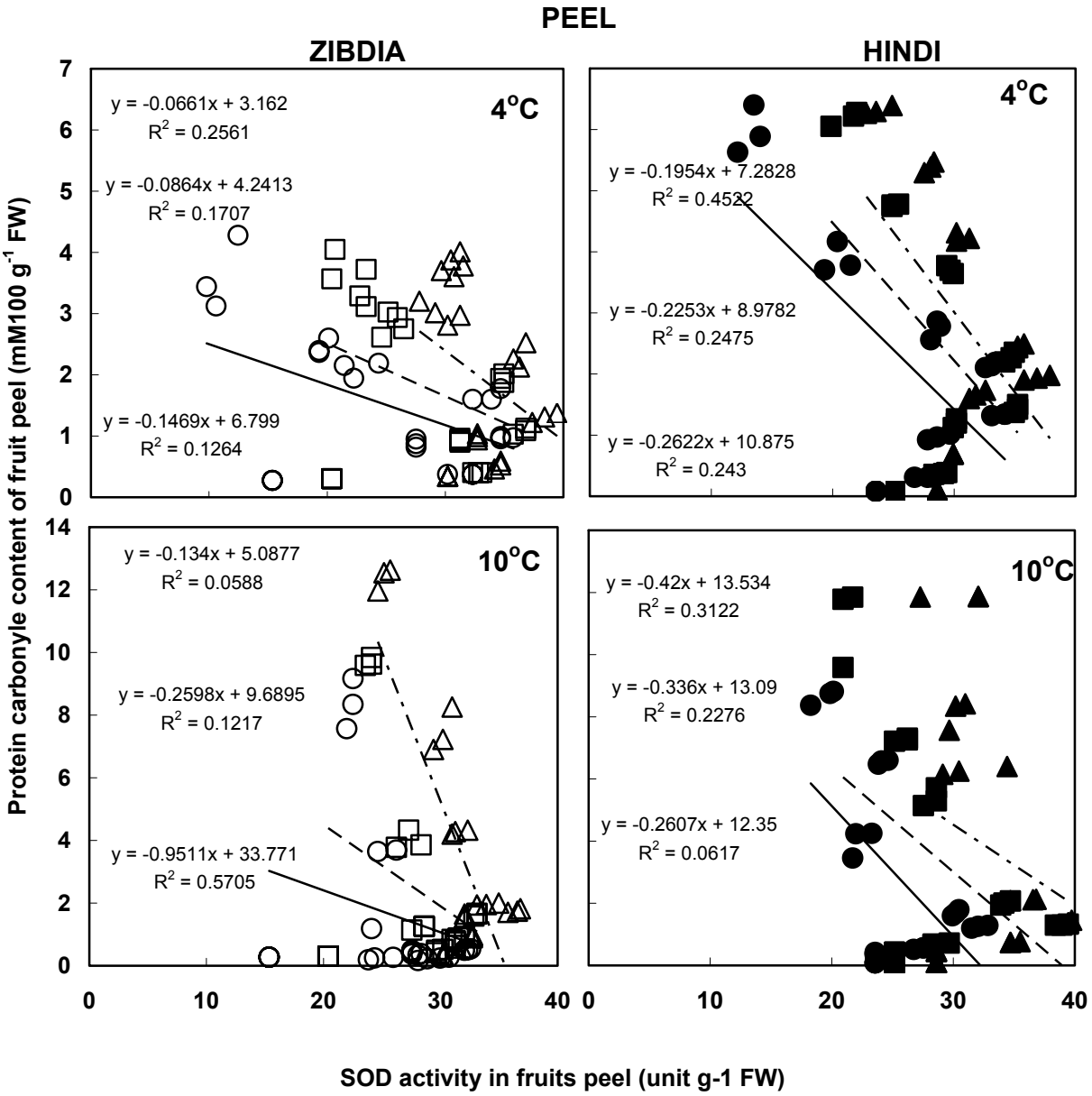

Figure 7a. The relationship protein carbonyl (PCG) content in fruits peel in function of superoxide dismutase (SOD) in fruits peel of two mango varieties Zibdia and Hindi were harvested in different maturity stages were stored at 4 and 10°C for 35 days. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

The accumulation of MDA equivalents correlates with the increase in electrolyte leakage that develops during cold storage (Chapter 3). Using data for the accumulation of MDA equivalents reported elsewhere (chapter 4), the relationship between membrane oxidation, and the activity of anti-oxidant enzymes can be explored (Figure 10, 11, 12 and 13). It is evident that APX activity is in broad terms inversely correlated with the accumulation of MDA equivalents (Figure 10).

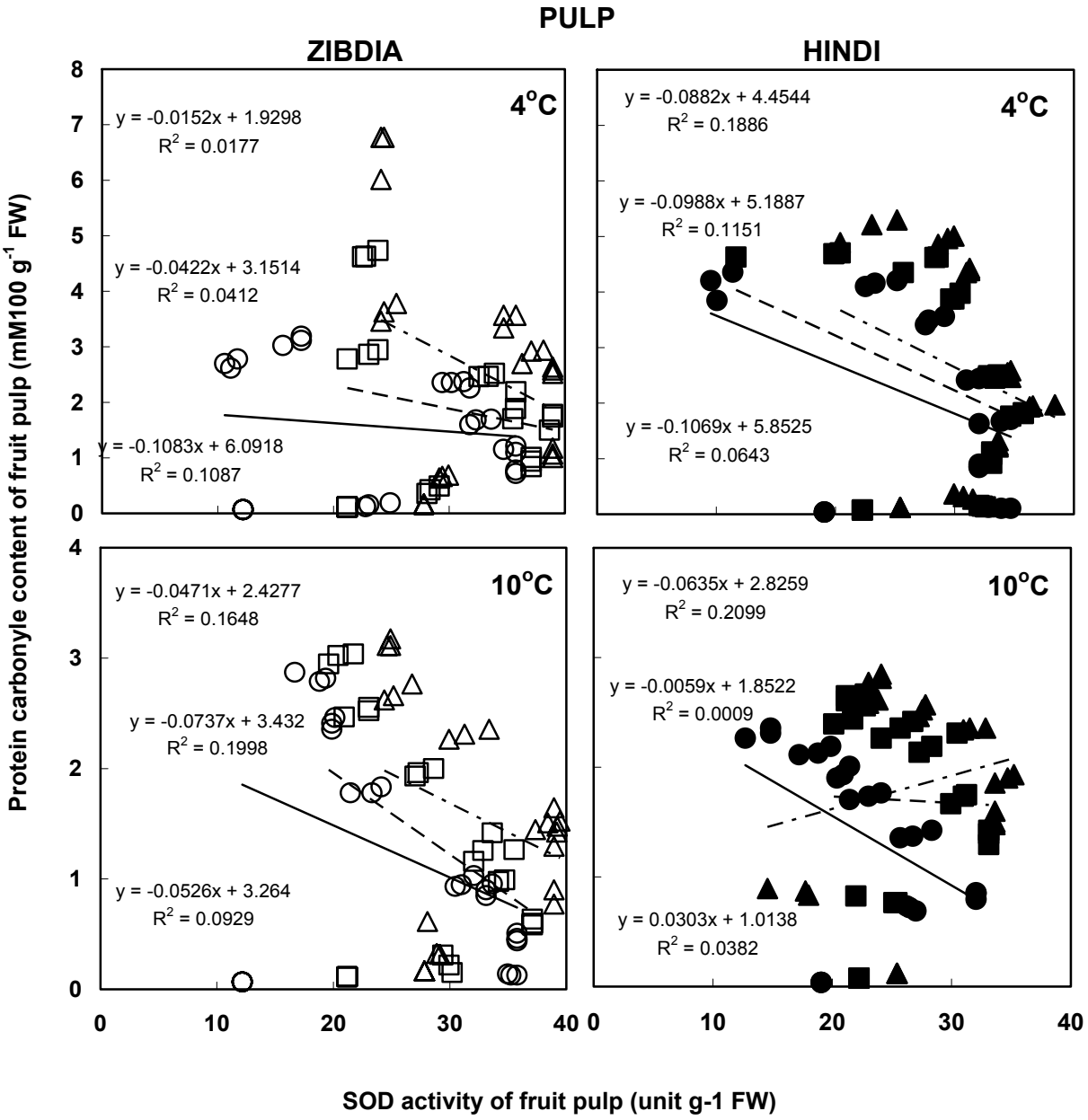

Figure 7b. The relationship protein carbonyl (PCG) content in fruits pulp in function of superoxide dismutase (SOD) in fruits pulp of two mango varieties Zibdia and Hindi were harvested in different maturity stages were stored at 4 and 10°C for 35 days. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

However in detail the correlation is often weak, especially in the pulp. With catalase activity the correlation with the accumulation of MDA equivalents is clearer, especially in the peel. Both CAT and APX act to destroy peroxide, though acting by different mechanisms: CAT acts alone, whereas APX requires ascorbic acid.

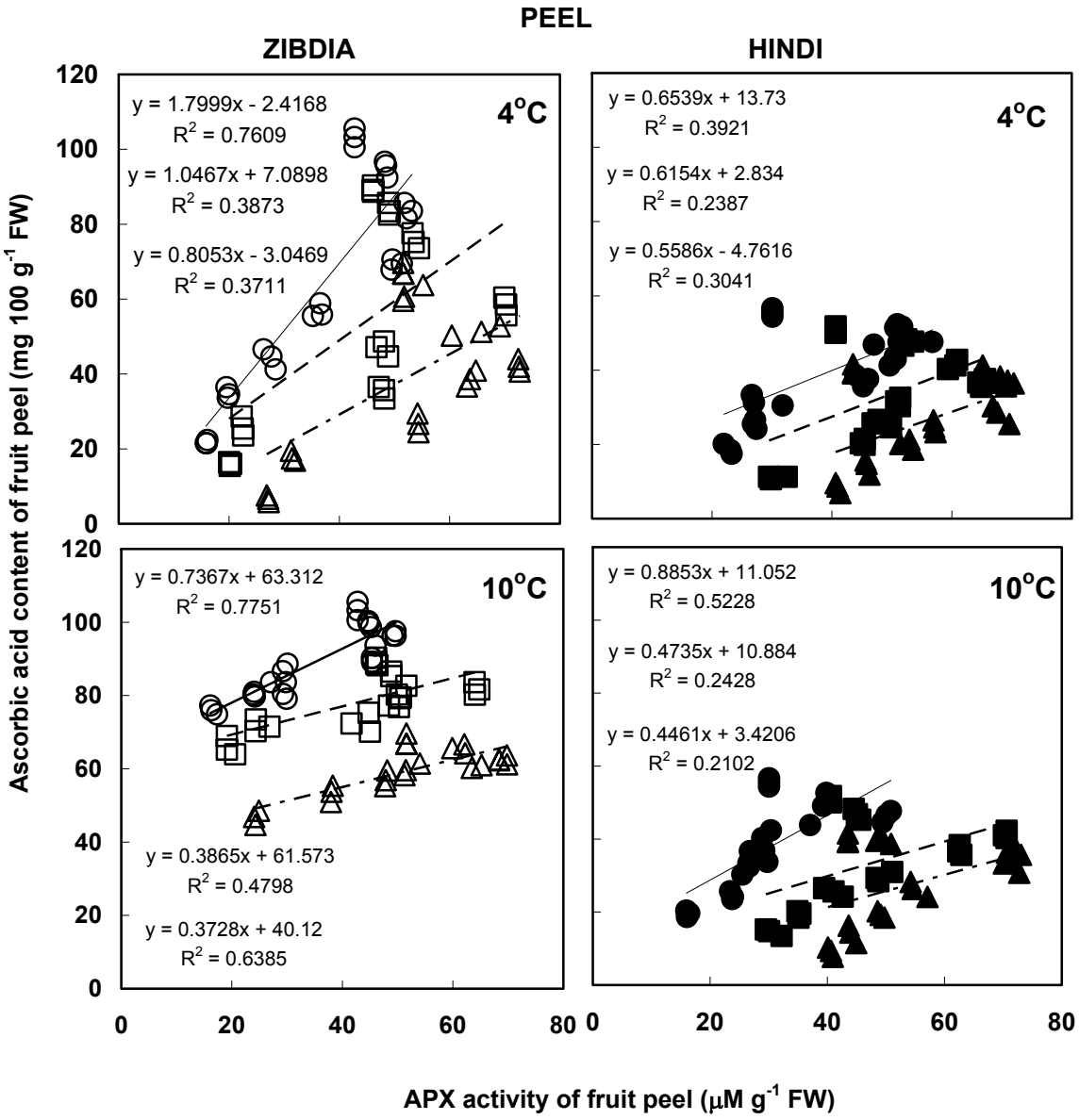

Figure 8a. The relationship of ascorbic acid content in function of ascorbate peroxidase (APX) activity on fruits peel of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

Ascorbic acid levels are closely inversely correlated with the accumulation of MDA equivalents. This could be interpreted as evidence that the protective, anti-oxidant property of APX is limited by ascorbic acid supply, however it must be borne in mind that ascorbic acid has varied functions in anti-oxidative metabolism (e.g. regeneration of oxidized tocopherol) and it may be another function that is the source of the good correlation between ascorbic acid and the accumulation of MDA equivalents. The accumulation of MDA equivalents appears to be largely independent of SOD activity in either the peel or the pulp. This would imply that changes in SOD activity play no major role in the increase of membrane oxidation.

This is not to say that SOD activity is not important: SOD is a required for aerobic life, but it may be that the oxidative stress leading to membrane damage has not relation to superoxide formation, or it may mean that SOD was present in excess. It could also be that a specific form of SOD in a specific cell-compartment was would have a correlation with MDA equivalent formation.

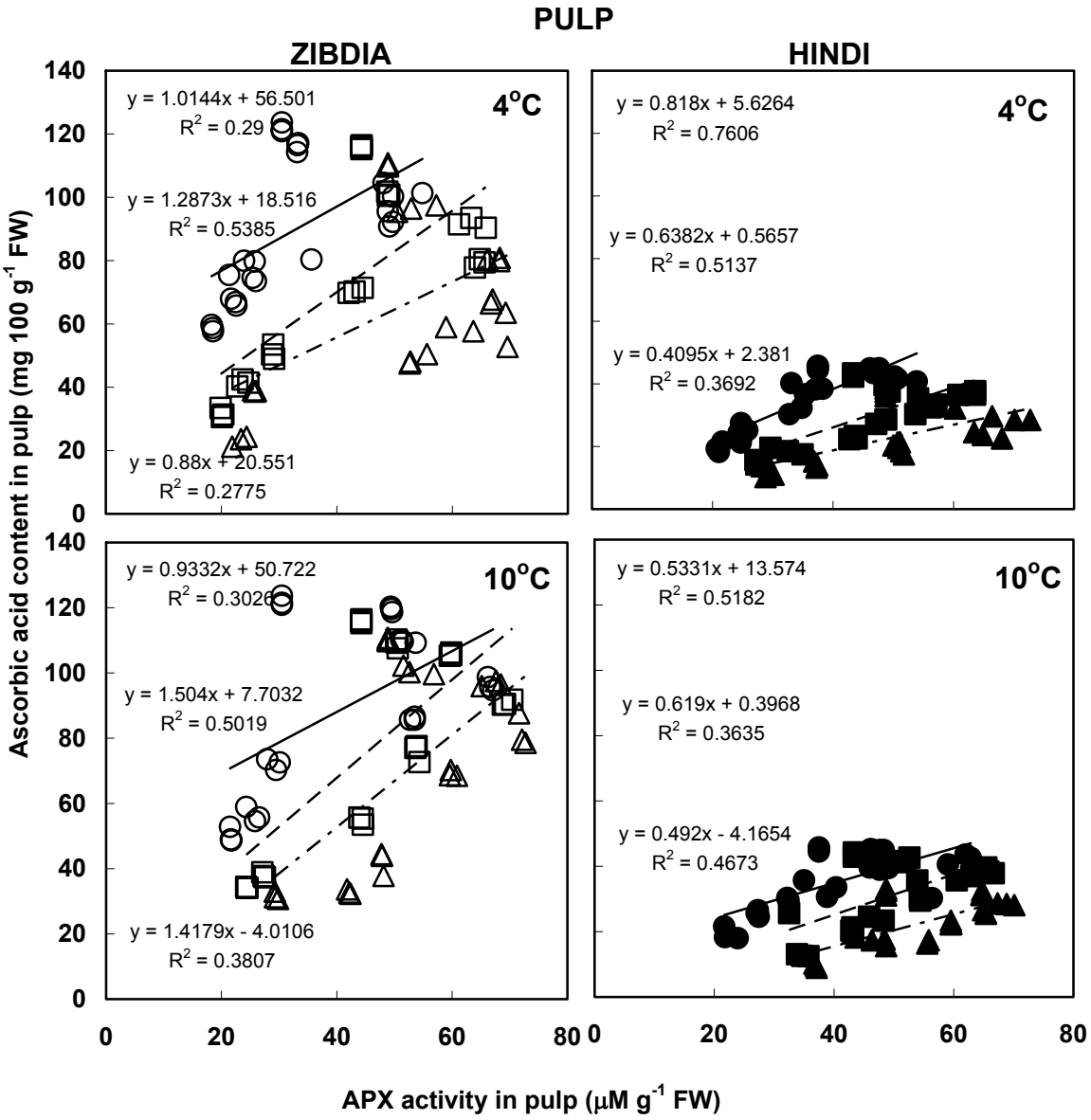

Figure 8b. Presents the relationship of ascorbic acid content in function of ascorbate peroxidase (APX) activity on fruits pulp of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

As with SOD, GR activity likewise has no correlation with the accumulation of MDA equivalents, and as with the SOD this may imply that the oxidative syndrome

leading to oxidation of fatty acids is not affected by GR activity, or that GR activity was saturating.

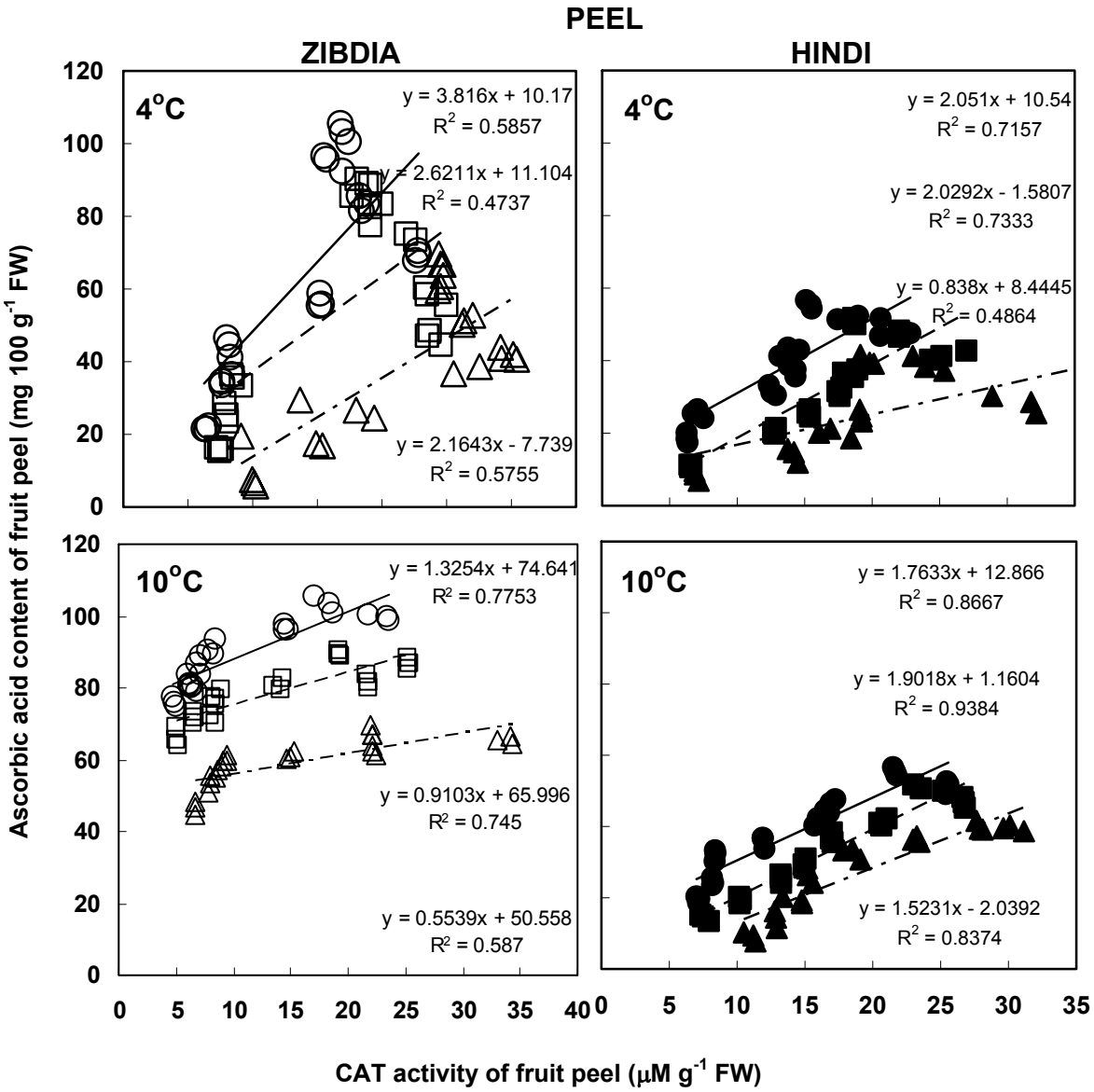

Figure 9a.Presents the relationship of ascorbic acid content in function of catalase (CAT) activity on fruits peel of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

It may also be that as with APX and ascorbate, the levels of glutathione might limit the anti-oxidative activity of GR. It has earlier been shown that protein carbonyl formation is closely correlated with the accumulation of MDA equivalents. This being so, it is evident that similar correlations would be expected between protein carbonyl formation and anti-oxidative enzyme activity. In both the peel and the pulp the activity of CAT has a good inverse correlation with protein carbonyl formation

(Figure 6), whereas with APX this correlation is weaker, and with SOD it is very poor (Figure 7).

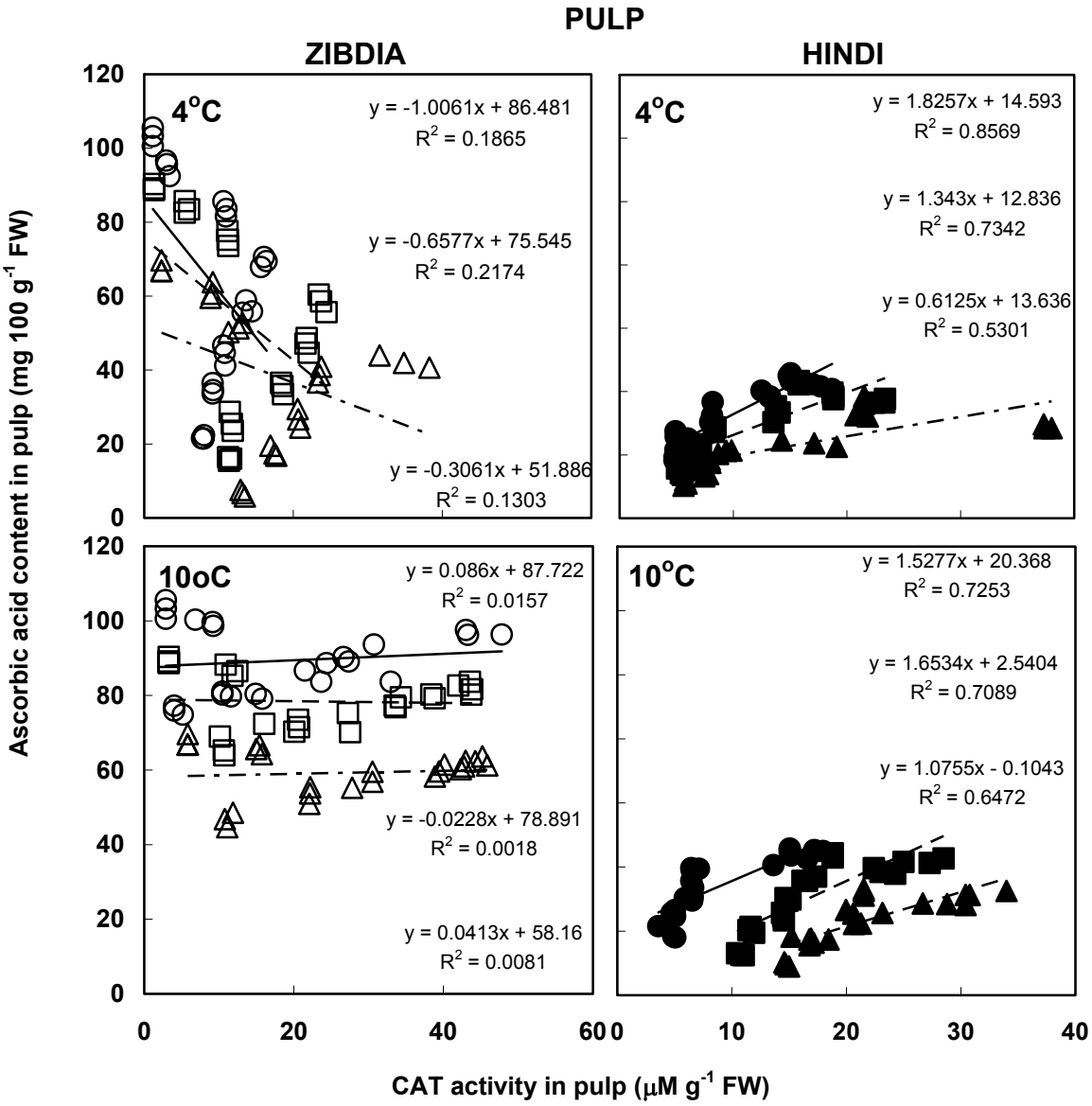

Figure 9b. The relationship of ascorbic acid content in function of catalase (CAT) activity on fruits pulp of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

Another index of cell injury is ion leakage, which is a commonly used technique to assess cell damage or viability. The detail of the effect of storage temperature on the increase of ion leakage with time has been described previously (Chapter 3). Ion leakage correlates well with the accumulation of MDA equivalents (Chapter 4), and as MDA equivalents are formed from membrane oxidation this link may be direct. In spite of the expected similarity in the correlations obtained between

enzyme activity and MDA equivalents, it was thought valuable to consider how changes in anti-oxidative enzyme activity might correlate with ion leakage.

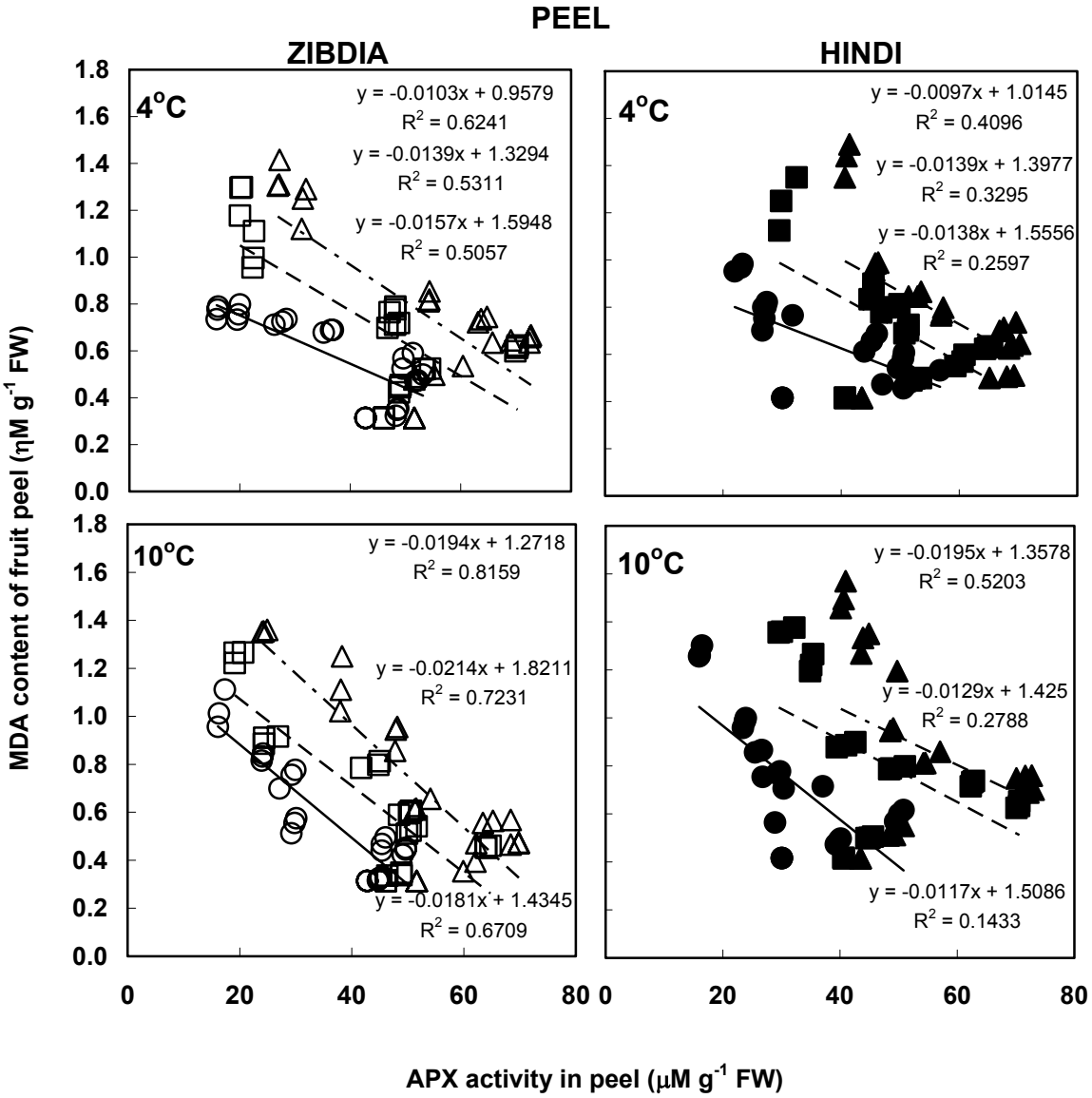

Figure 10a. The relationship of malondialdehyde (MDA) content in function of ascorbate peroxidase (APX) activity on fruits peel of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

The inverse correlation between ion leakage and CAT activity is good, especially for Hindi peel and pulp tissues (Figure 15). Likewise the correlation between APX activity and ion leakage was also good (Figure 14); comparable to that shown between APX activity and the accumulation of MDA equivalents, with decreased activities of APX correlating with increased leakage (Figure 16 and 17). The activity

of neither GR nor SOD correlated with the increase of ion leakage that occurred during storage.

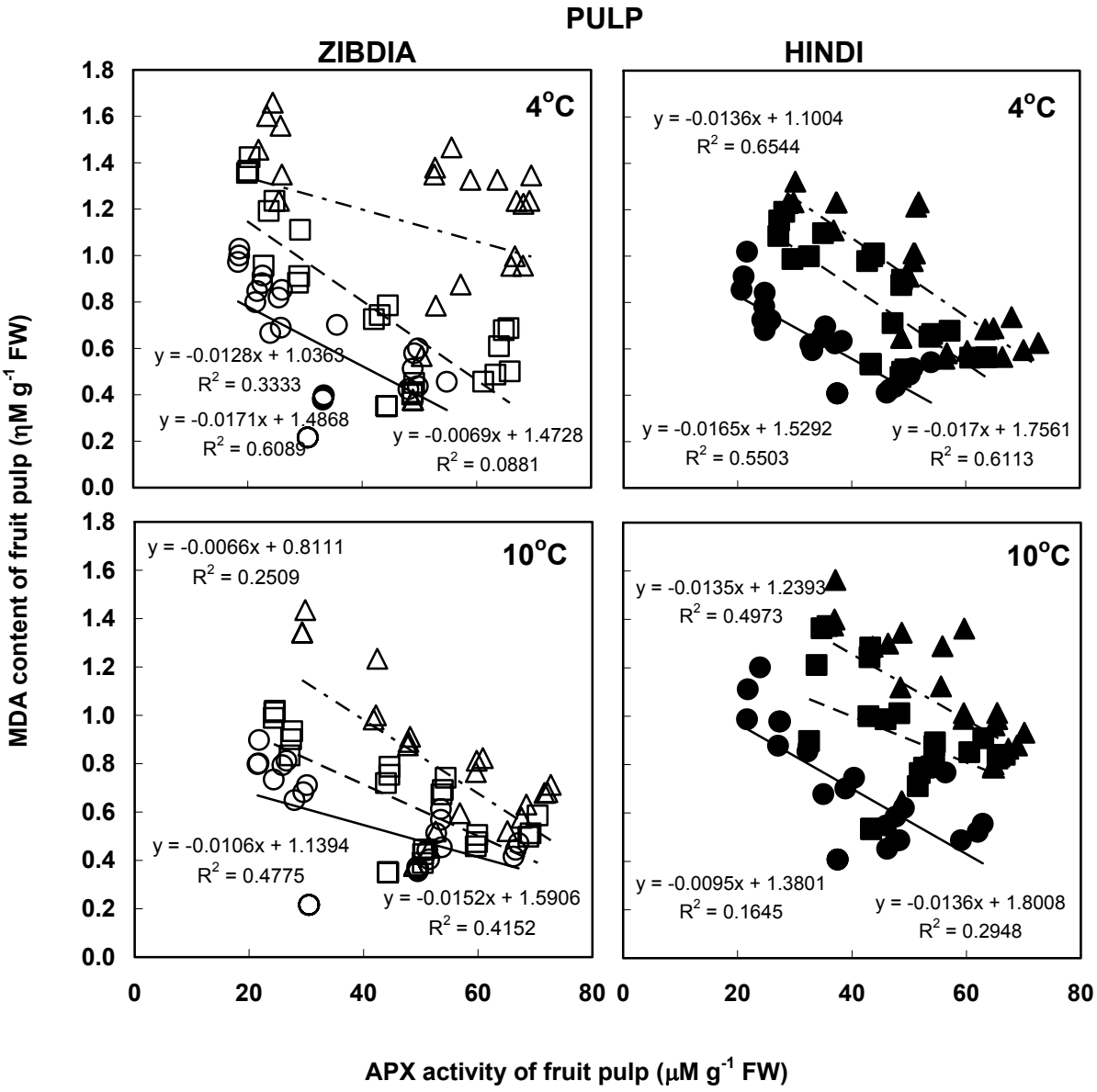

Figure10b. The relationship of malondialdehyde (MDA) content in function of ascorbate peroxidase (APX) activity on fruits pulp of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

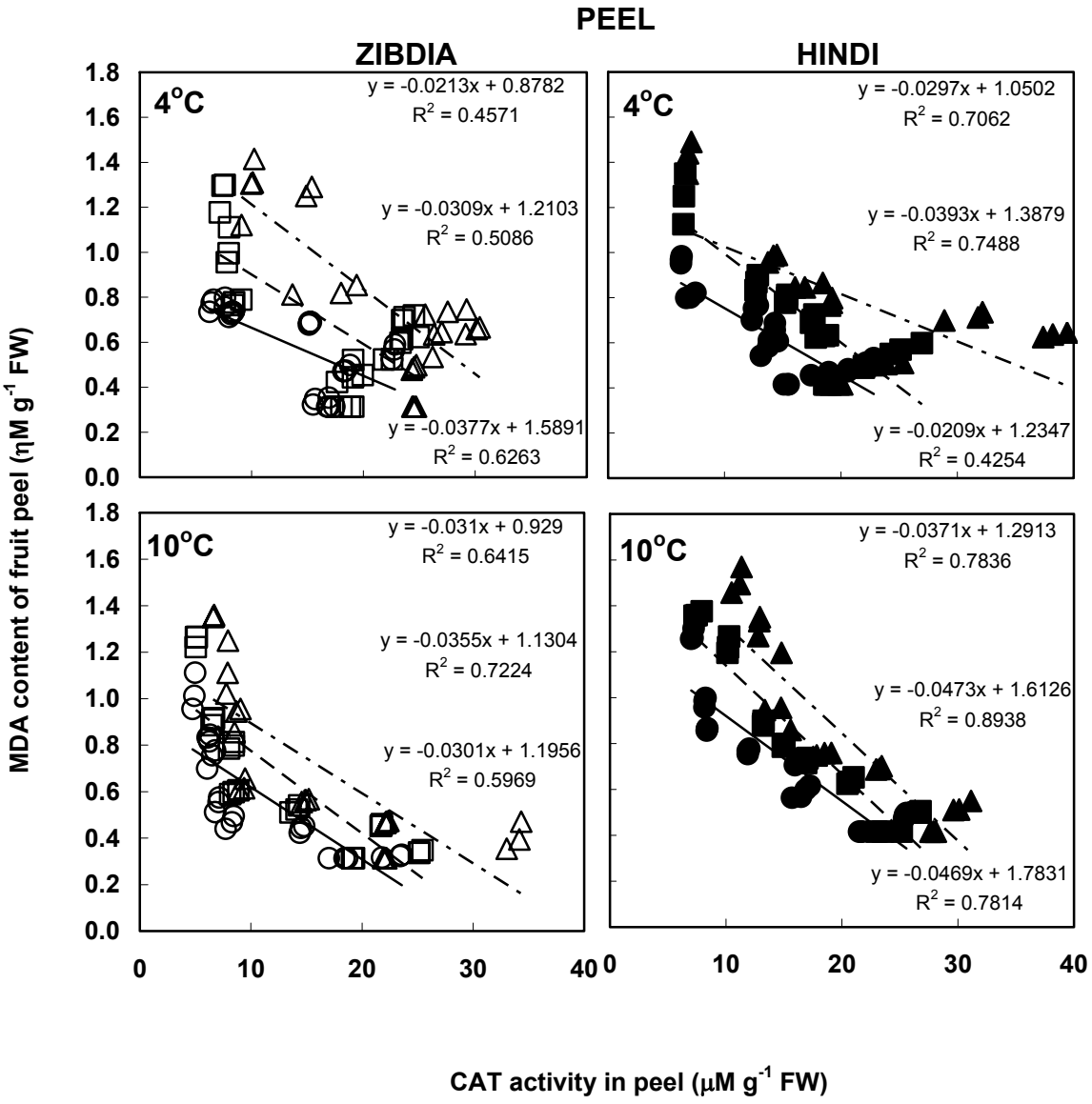

Figure 11a. Presents the relationship of malondialdehyde (MDA) content in function of Catalase (CAT) activity on fruits peel of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

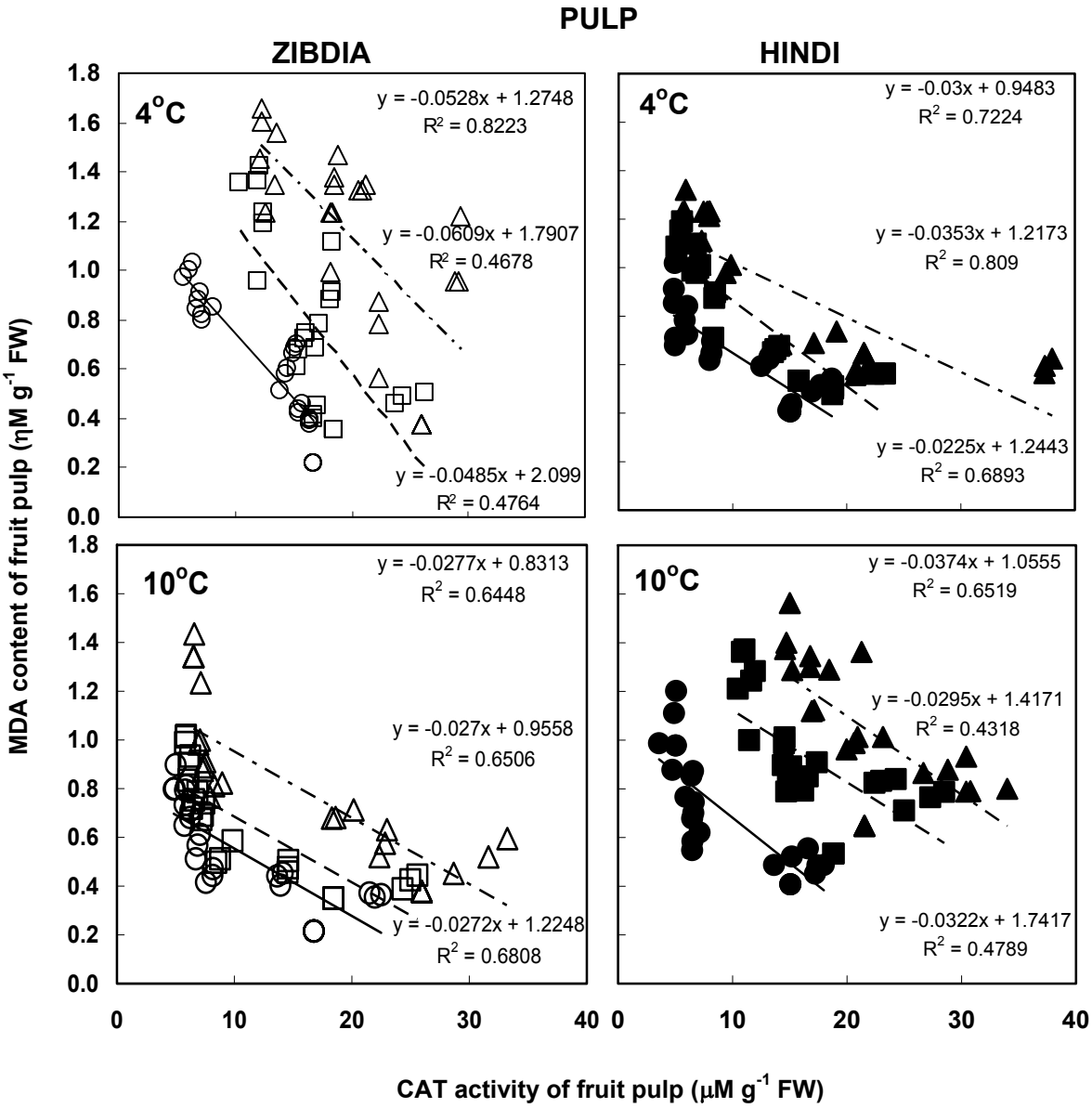

Figure 11b. The relationship of malondialdehyde (MDA) content in function of Catalase (CAT) activity on fruits pulp of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

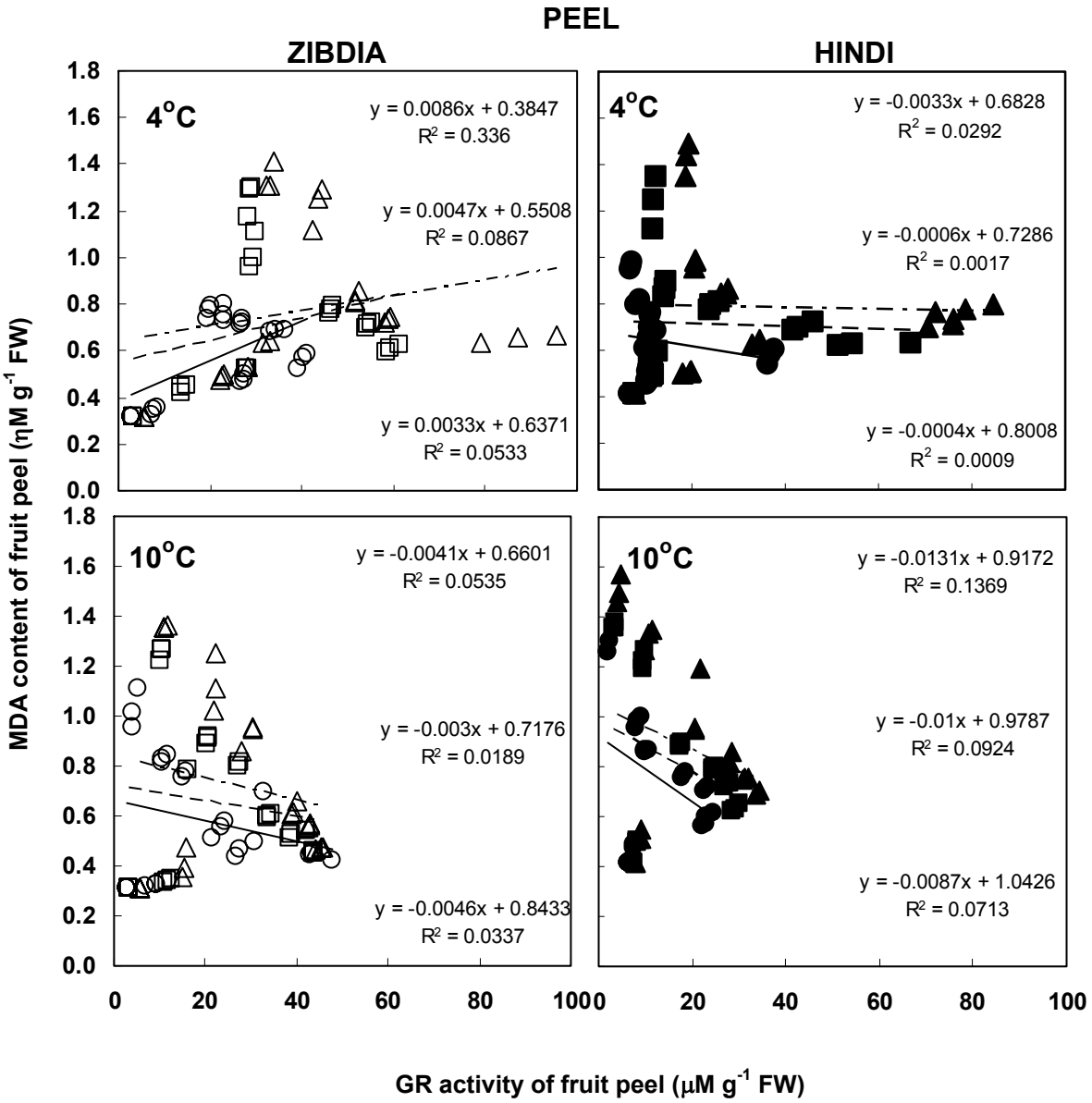

Figure 12a. The relationship of malondialdehyde (MDA) content in function of glutathione reductase (GR) activity on fruits peel of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

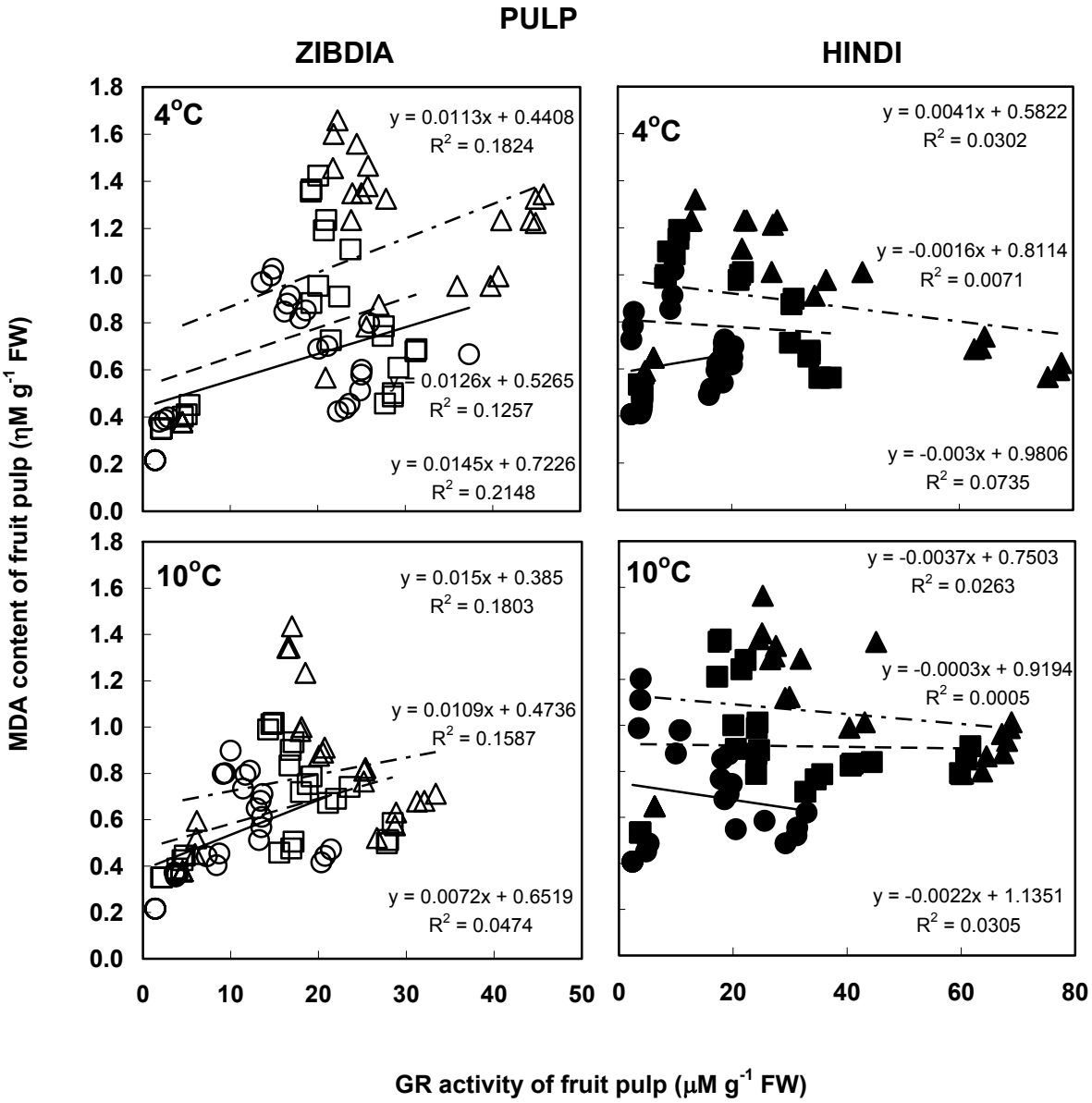

Figure 12b. The relationship of malondialdehyde (MDA) content in function of glutathione reductase (GR) activity on fruits pulp of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

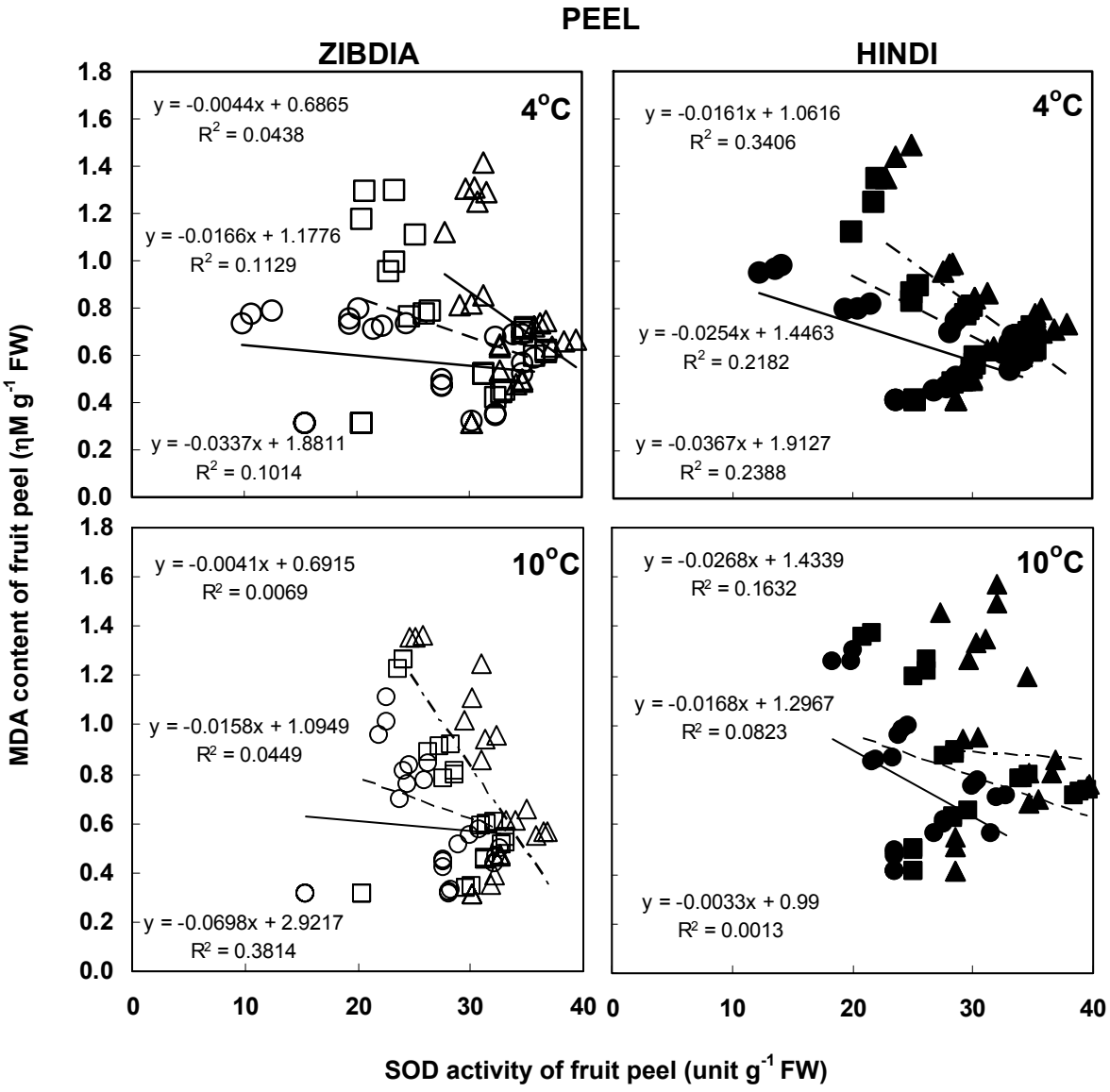

Figure 13a. The relationship of malondialdehyde (MDA) content in function of superoxide reductase (SOD) activity on fruits peel of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

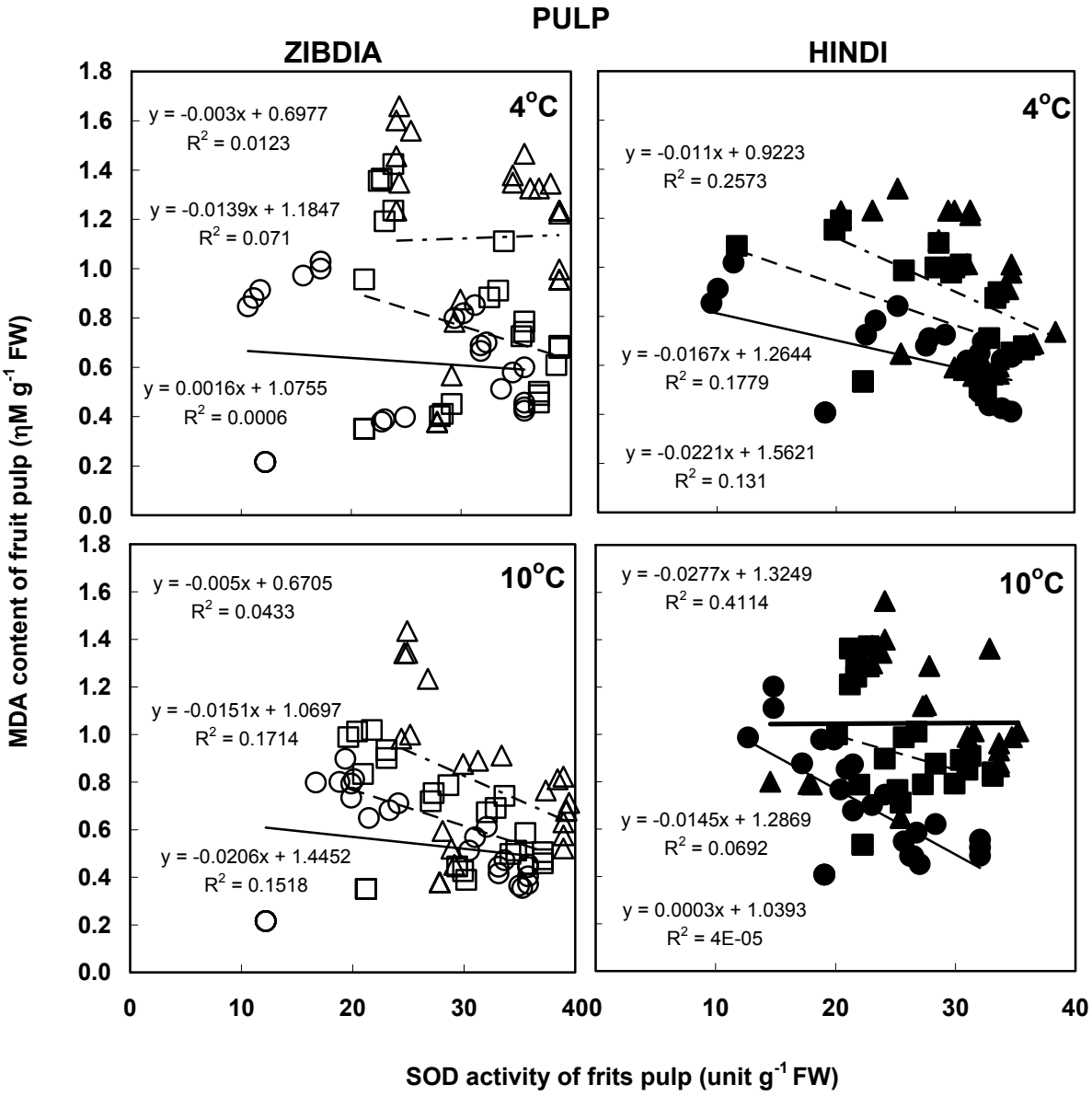

Figure 13b. The relationship of malondialdehyde (MDA) content in function of superoxide reductase (SOD) activity on fruits pulp of Zibdia and Hindi varieties harvested in different maturity stages were stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3). Solid lines represent linear regression.

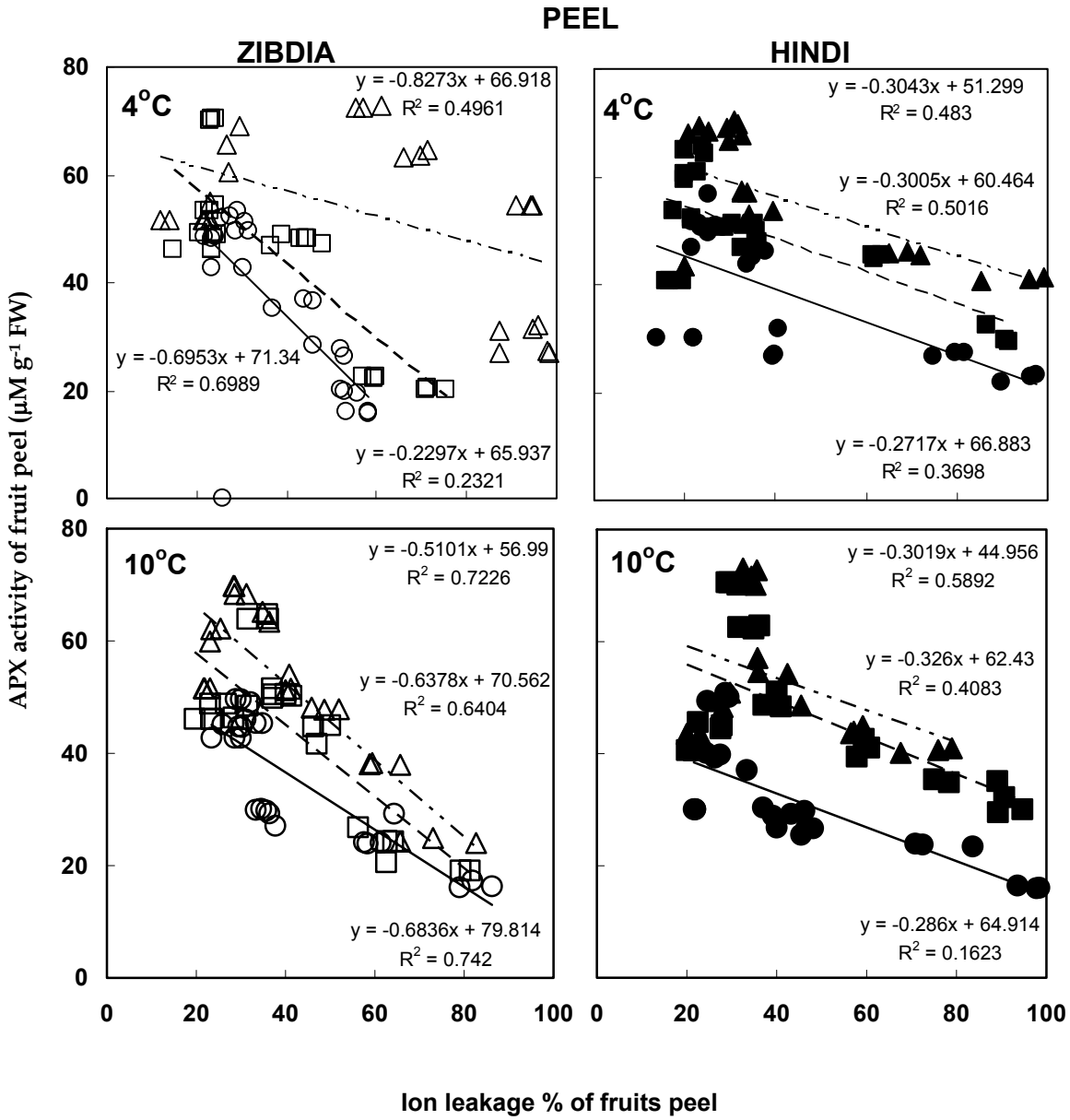

Figure 14a. The linear relationship of ascorbate peroxidase (APX) activity in function of ion leakage percentage of fruits peel of two mango cultivars 'Zibdia' and 'Hindi' harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Enzymes activity measured on peels, versus storage time at 5 different storage temperatures. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - λ - M1, - ν - M2, and -▲- M3). The lines represent linear regression.

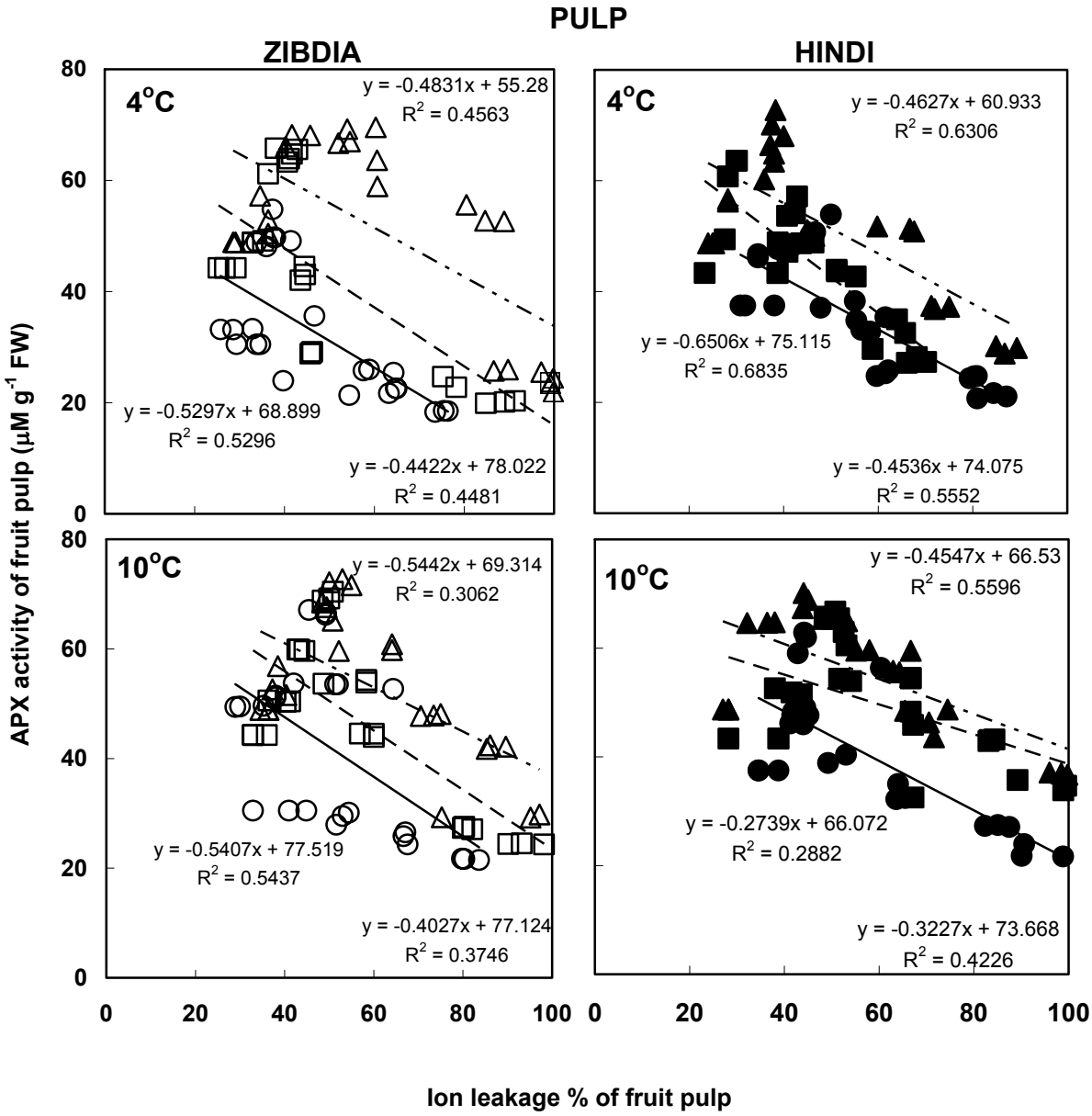

Figure 14b. The linear relationship ascorbate peroxidase (APX activity in function of ion leakage percentage of fruits peel of two mango cultivars 'Zibdia' and 'Hindi' harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - λ - M1, - ν - M2, and -▲- M3). The lines represent linear regression.

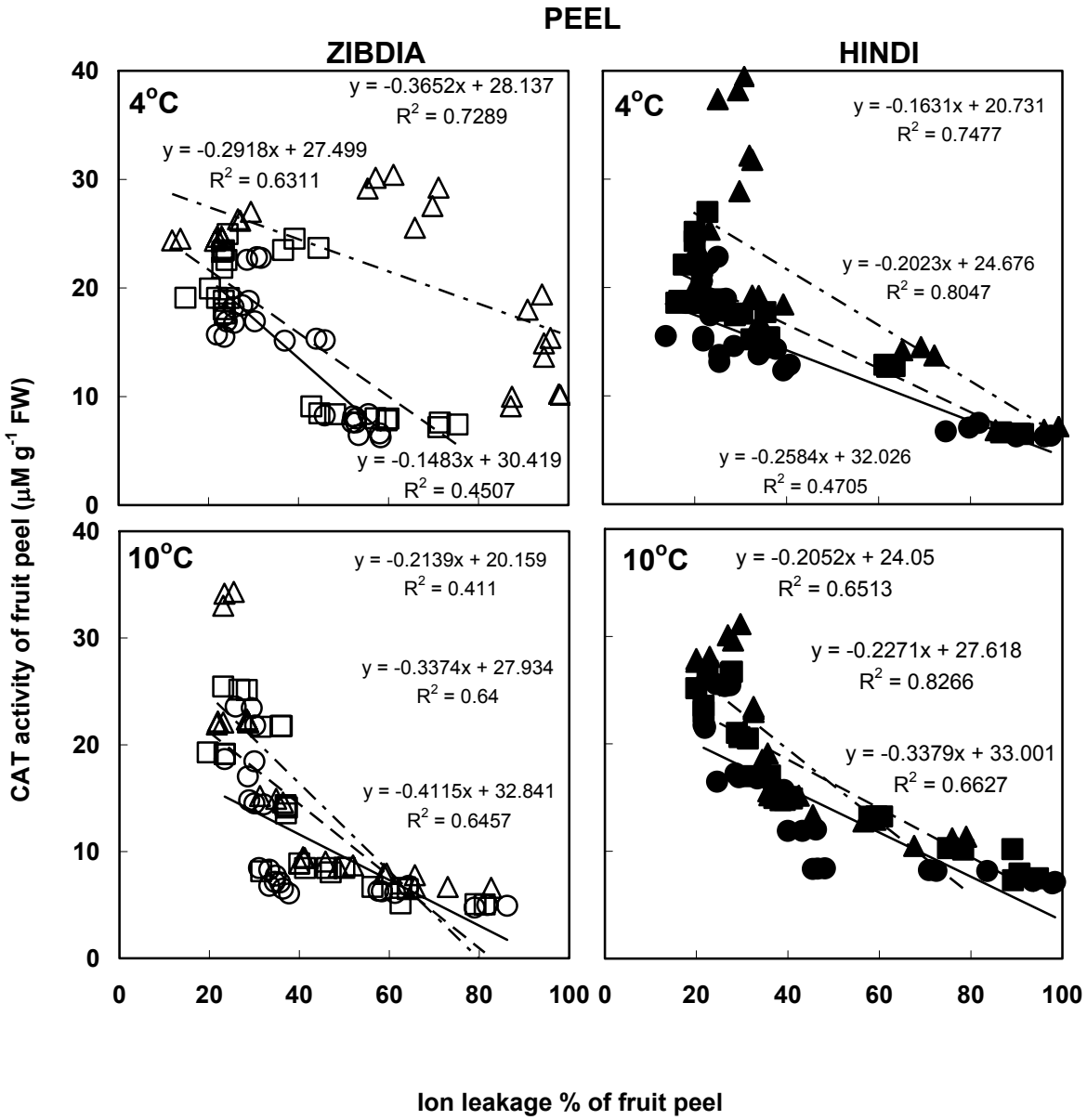

Figure 15a. The relationship of Catalase (CAT) activity in function of ion leakage percentage of fruit peel of two mango cultivars 'Zibdia' and 'Hindi' harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - λ - M1, - ν - M2, and -▲- M3). The lines represent linear regression.

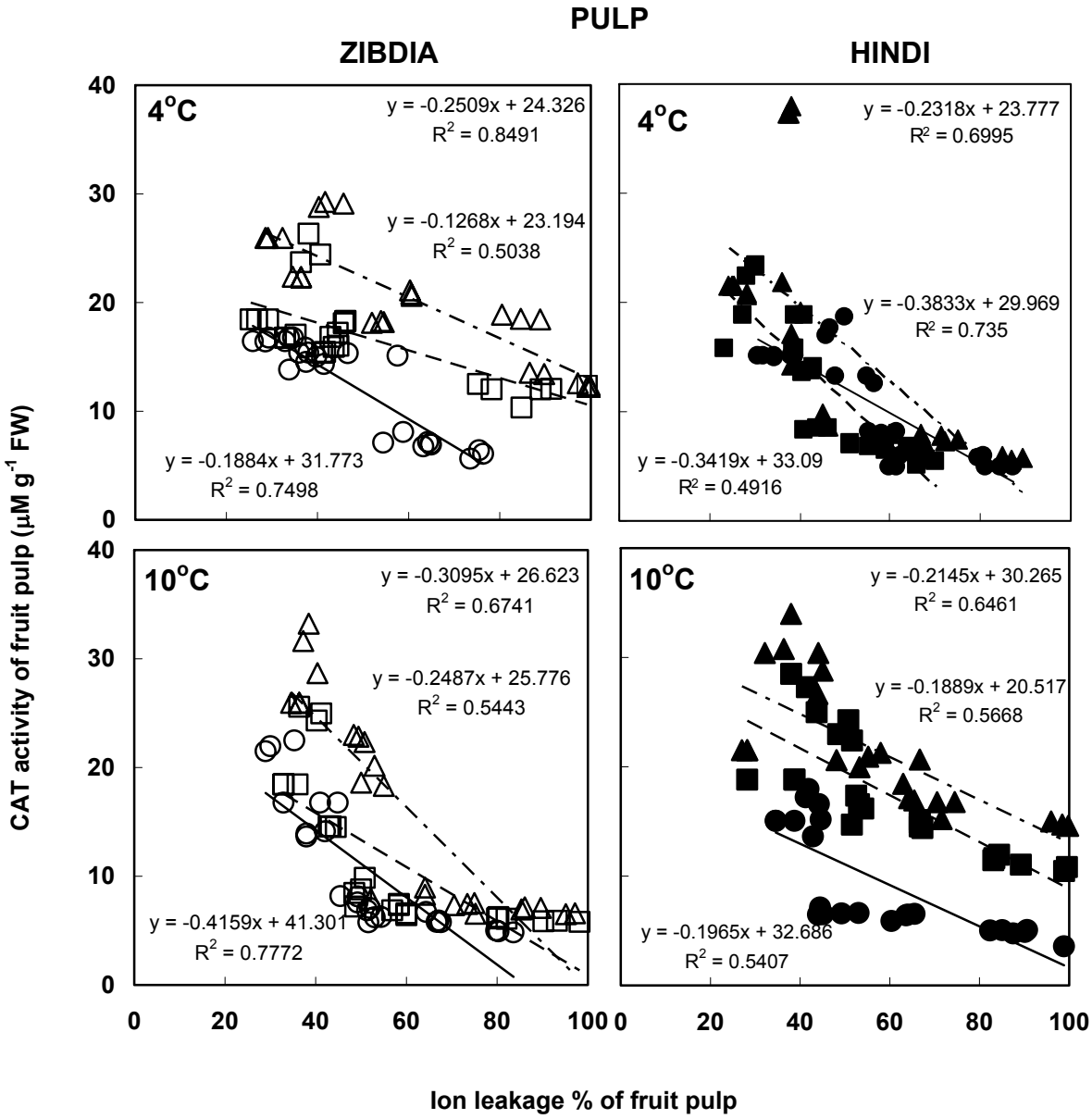

Figure 15b. The relationship of Catalase (CAT) activity in function of ion leakage percentage of fruits pulp of two mango cultivars 'Zibdia' and 'Hindi' harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - λ - M1, - ν - M2, and -▲- M3). The lines represent linear regression.

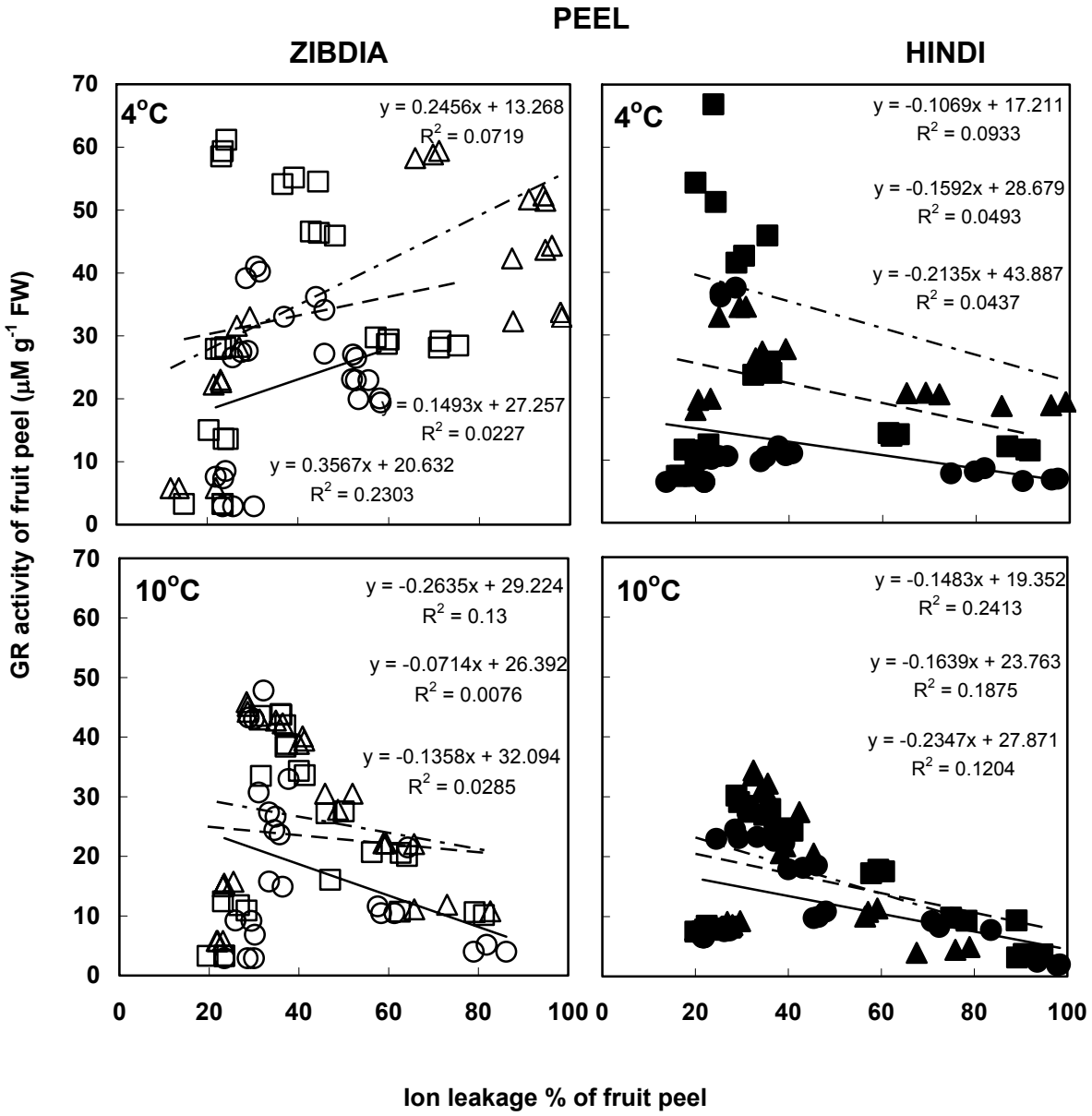

Figure 16a. The relationship Glutathione reductase (GR) activity in function of ion leakage percentage of fruits peel of two mango cultivars 'Zibdia' and 'Hindi' harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - λ - M1, - ν - M2, and -▲- M3). The lines represent linear regression.

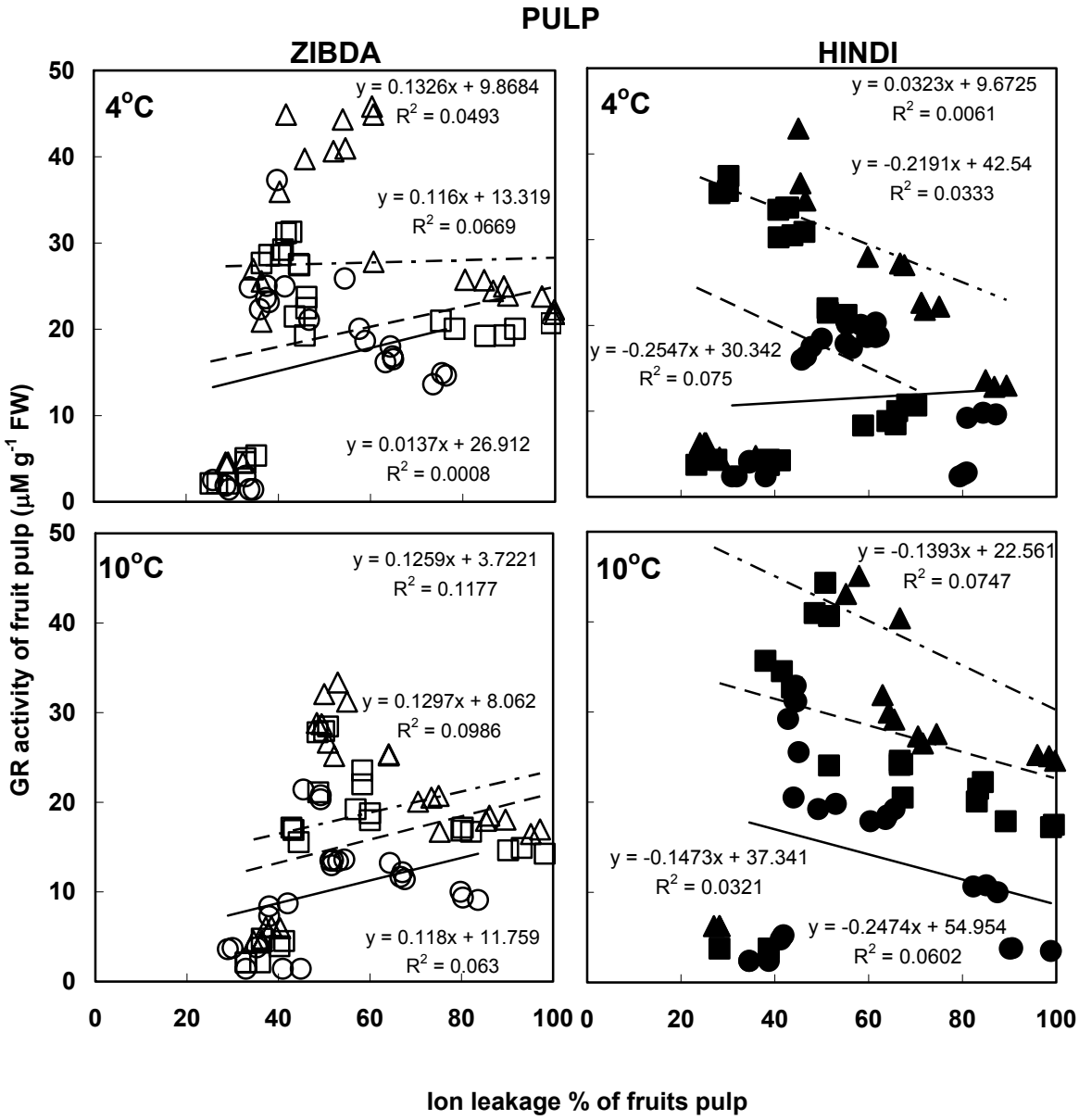

Figure 16b. The relationship of Glutathione reductase (GR) activity in function of ion leakage percentage of fruits pulp of two mango cultivars 'Zibdia' and 'Hindi' harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - λ - M1, - ν - M2, and -▲- M3). The lines represent linear regression.

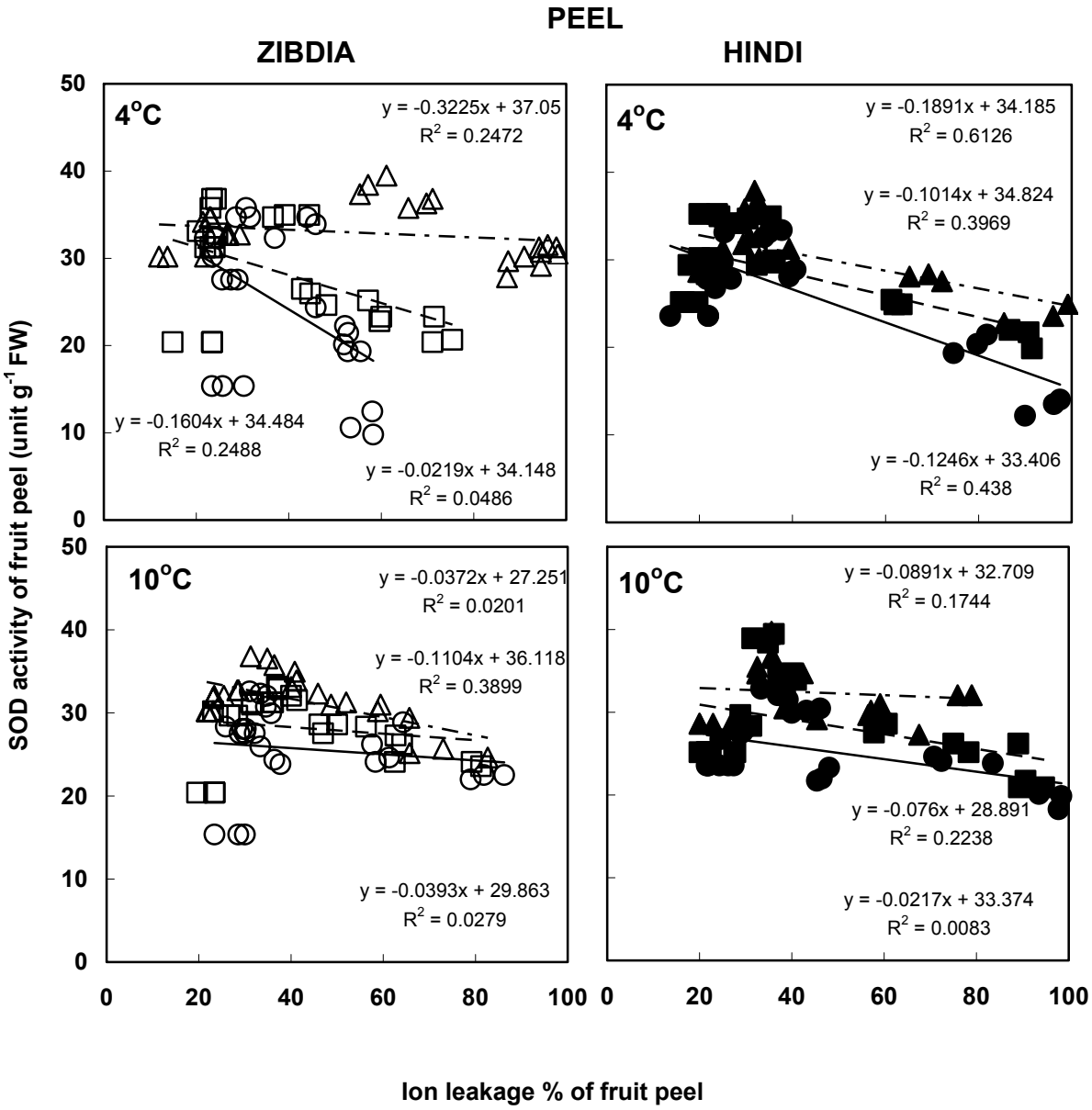

Figure 17a. The relationship of Superoxide dismutase (SOD) activity in function of ion leakage percentage of fruits peel of two mango cultivars 'Zibdia' and 'Hindi' harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - λ - M1, - ν - M2, and -▲- M3). The lines represent linear regression.

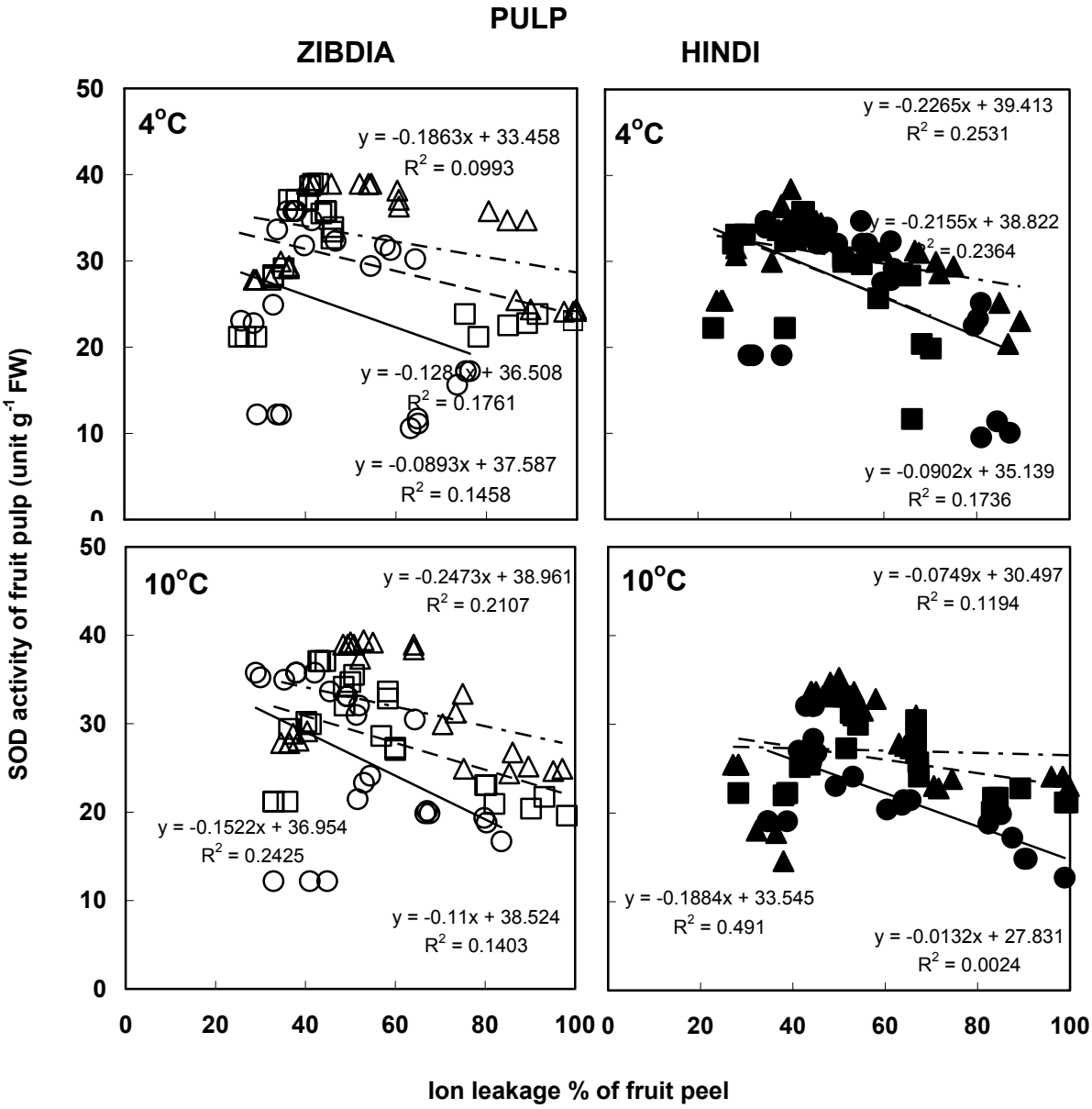

Figure 17b. The relationship of Superoxide dismutase (SOD) activity in function of ion leakage percentage of fruits pulp of two mango cultivars 'Zibdia' and 'Hindi' harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), stored at 4 and 10°C for 35 day. Symbols represent the different maturity stages are (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - λ - M1, - ν - M2, and -▲- M3). The lines represent linear regression.

Acknowledgment

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Chapter 6

On the Ethylene production, respiration rate, firmness and ion leakage in mango fruits

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Abstract

Physiological changes such as: ethylene production, respiration rate, firmness, and ion leakage were studied on two mango varieties (*Mangifera indica* L. 'Zibdia' and 'Hindi Be-Sennara'). These changes were monitored at 3 different harvest maturity stages for fruits stored for up to 35 days at different temperatures (2, 4, 7, 10, and 20^{o}C). C_2H_4 production increased to a maximum production rate after the 5th day of storage. The climacteric respiration showed a peak after day 10. Fully mature (M3) fruits produced more ethylene and respiration compared to immature (M1) and half mature fruits (M2). Thereafter they decreased until day 35. Hindi fruits show higher firmness than Zibdia fruits. Both mango varieties show higher firmness at low storage temperature (2 or 4^{o}C) compared with fruits stored at 7 or 10^{o}C. Ion leakage in Zibdia fruits was higher than Hindi fruits when the fruits were stored at 2 or 4^{o}C compared with fruits stored at 7 or 10^{o}C. Fruit pulps showed higher ion leakage than peel. Decreased firmness and increased ion leakage due to ripening processes when fruits were stored at 20^{o}C which are related to changes in cell lipid membrane. Physiological changes are induced by the severity effects of low storage temperatures at different storage times. At lower temperature, storage induced CI in both varieties as an effect of generation active oxygen species (AOS). These AOS react with cell lipid membranes and initiate lipid peroxidation. Because of these processes, chilling injury will result which affects the quality elements of mango fruits.

Keywords: chilling injury (CI); Ethylene (C_2H_4); Respiration (CO_2); Firmness (Fr); Electrolyte leakage (EL)

1. Introduction

Mango is one of the most popular fruits worldwide (Mitra and Baldwin 1997) and also very popular in Egypt (Mamdouh, 1997). It is considered to be a climacteric fruit which ripens rapidly after harvest, and to be a moderate producer of C_2H_4 and CO_2 (Kader, 2002). Disease susceptibility, sensitivity to low storage temperature below 12^oC, and perishability due to ripening and softening limit the storage, handling and transport potential of the fruit (Mitra and Baldwin, 1997). Avoidance of prolonged exposure to low temperatures is considered an important requirement for this fruit as it is chilling sensitive. As a result of this sensitivity, low temperatures produce a range of physiological and metabolic disorders that lead to serious quality losses. The various dysfunctions that arise under low temperature conditions result in various physical and metabolic changes that are easily scored and which can therefore be used to assess the degree of chilling injury.

Mango is a climacteric fruit. Measurements on the mango variety 'Manila' showed that a typical climacteric respiration peak develop by the 6th day of storage and decreased steadily thereafter (Dinora et al., 1997). Other climacteric respiratory patterns have been observed in the varieties 'Tommy Atkins' by Mitcham and McDonald, (1992), and 'Kent' by Trinidad et al. (1997). The maximum of C_2H_4 evolution occurred simultaneously with the increase of respiration in the variety 'Haden' (Trinidad et al., 1997). On the other hand, McCollum (1993) found that for the variety 'Kent' C_2H_4 production reached a maximum after the climacteric of respiration. These results indicated that the degree of synchronization of C_2H_4 evolution with the respiratory climacteric depends upon the mango variety.

Various factors influence the production of ethylene. Low temperatures during long term storage inhibit C_2H_4 production in fruits, including mangoes (Valero et al., 1997). Besides this, the maturity of mango fruits affects the rate of C_2H_4 production: fully mature fruits produce larger amounts of C_2H_4 than immature and half mature fruits (Majeed and Jeffery, 2002). Physiologically, the effects of ethylene on the ripening and senescence of plants is correlated well with climacteric respiration and changes in many membrane parameters, such as loss of phospholipids and increased membrane permeability (Noodén and Leopold, 1988). The decrease of climacteric respiration rates at low storage temperatures is a result of decreased metabolic activity. This is considered a secondary response to low temperatures. These changes in C_2H_4 production and respiration rates are correlated with major changes in the properties of the fruits. For example, various hydrolytic enzymes that degrade cell wall components are released and their activity is correlated with changes in fruit tissue firmness (Ketsa et al., 1999b). Overall, changes in fruit and vegetable firmness are a consequence of physical, physiological, and chemical changes that occur in the tissues of the product. The processes associated with

changes in firmness have been intensively studied because firmness is considered to be an important factor determining the consumer acceptability of fruits. Fruit firmness is a set of physical characteristics that are sensed by means of touch, and are related to the deformability or disintegration of the fruit tissues, and the flow of tissue fluids in response to the application of a force. It is possible to measure the response of a fruit to an applied force in terms of the time dependent development of a deformation and use this to describe objectively the phenomena that result in the sensory evaluation of firmness (Bourne, 1980).

The processes that changes firmness, and thus product itself firmness, are influenced by the product's history and immediate environment. For example, storage temperature is one of the factors that affects changes in the firmness of 'Keitt' mango fruits. Lederman et al., (1997) found that storage of these fruits at low temperature improved the firmness. Owing to their chilling sensitivity, low storage temperature produces an increased membrane permeability in mango. Firmness, however, depends not only on cell-wall properties, which are often studied, but also on membrane integrity. Without membrane integrity it is impossible to generate the turgor forces that give rise to many of the mechanical properties of thin-celled tissues, such as fruit pulp. So, an aim of this study was to see if damage to the cell membranes of the pulp tissue resulting from storage at low temperatures was correlated with changes in tissue mechanical properties.

Tissue softening is one of the consequences of ethylene exposure. So, in addition to tissue firmness, we also measured changes in ethylene evolution and respiration to investigate the effect of storage at chilling temperatures on the climacteric of mangoes. These measurements were made on two varieties of mango: Zibdia and Hindi, which were harvested at three different maturity stages and stored at different temperatures for up to 35 days.

2. Materials and methods

2.1. Fruits and storage condition:

Zibdia and Hindi Be-Sennara mango fruits were harvested from trees more than 20 years old. (Details of harvesting, transporting and shipping are given previously in Chapter 3). Upon arrival in PPO, the fruits were divided into 5 batches; each one composed of 216 Zibdia and 216 Hindi (72 fruits of each maturity stage). These batches were stored at 2, 4, 7, 10 and 20^{o}C in darkness. Samples of 10 fruits were taken from each temperature every 5 days (up to 35 days) and used for measurements of CO_2 and ethylene evolution, electrolyte leakage, the accumulation of malondialdehyde equivalents, and tissue firmness.

2.2. Ethylene production and respiration rate:

Ethylene and respiration rates of Zibdia and Hindi fruits were measured on samples of 9 fruits after 5 days interval for each maturity stage. Each sample was divided into 3 replicates of 3 fruits. Individual fruits were inserted in 1-liter glass jars, and incubated for 1h at each storage temperature. A sample of headspace atmosphere was collected and analyzed for ethylene and carbon dioxide by gas chromatography techniques (GC). These fruits were then used for destructive measurements (firmness and ion leakage). Ethylene was measured using a GC, GC 6000 Vega Series, Carlo Erba Instrument, (Milan, Italy) and carbon dioxide was also measured by GC techniques (Micro GC, Chrompack, Middelburg, The Netherlands)

2.3. Firmness measurement:

Fruit firmness was determined on the same fruits which were used previously in ethylene and respiration measurements. A Zwick® Universal Testing Machine with a 6.35-mm-diameter 60^{o} conical probe was used to perform these measurements. The instrument measures the force required for the mechanical probe to penetrate 5.5 mm into the tissue of fruits at a speed of 3 mm s^{-1}. The measurements were carried out according to the standard procedure, in the centre of the on two opposite faces of the fruit. The measured forces (in Newtons) were then averaged (Hofman et al., 1997).

2.4. Electrolyte Leakage

Changes in electrolyte leakage was described and measured in Chapter 3

2.5. Lipid peroxidation

Changes lipid peroxidation of cell membrane product (malondialdehyde; MDA) concentration was described and measured in Chapter 4

2.5. Statistical analysis

Measured data for ethylene, respiration, production, firmness and ion leakage were analyzed using analysis of variance (ANOVA). Four factors were included: time, temperature, variety and maturity stages. Means comparisons were undertaken using the least significant differences (L.S.D.) at p=0.05. Data were analyzed for two periods: the first is from 0-20 days, when all treatments were present and the second is from day 0-35 days for all treatments except for storage at 20^{o}C. Linear regression analysis and ANOVA were analyzed at the 5%

probability level. The statistical software package Genstat 8 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) was used.

3. Results

3.1. Ethylene production

Figure 1 shows the changes in ethylene production rate as a function of storage time (days) for both mango varieties at different storage temperatures (2-20^{o}C) for three maturity stages. Generally, the rate of ethylene production depends in a complex way on the storage time, temperature, variety and maturity stage. Both mango types show almost the same initial rate of ethylene production: approximately 10ml.kg^{-1}.h^{-1} at all the maturity stages. During storage in the temperature range 2-20^{o}C, ethylene evolution increases to a maximum that is higher than the initial rate of evolution by 2-7 times for Zibdia and 2-6 times for Hindi. The maximum is reached after 5 days of storage at all the temperatures and for all the maturity stages. For Zibdia the maximum ethylene evolution occurred in the 20°C fruits, and was substantially higher than the evolution from the fruits held at low storage temperatures. These latter fruits displayed a temperature sensitivity of the rate of maximum evolution with evolution increasing with increasing temperature. The effect of maturity stage on the climacteric of Hindi fruits was less marked than for Zibdia. In contrast to Zibdia, storage at 10°C did not decrease ethylene evolution during the climacteric compared to the fruits stored at 20°C, and with increasing maturity stage the rate of evolution from the 7°C fruits increased to that of the 10° and 20°C fruits. Evolution from the 2° and 4°C fruits was similar to each other and increased with maturity stage.

The maximum of ethylene evolution was followed by a period of decreasing ethylene production that differs depending mango type, and storage temperature. The ethylene evolution of Zibdia fruits stored at low temperatures decreased in parallel, with little difference between the temperatures, to a very low level at day 35. These differences parallel the variation in the maximum ethylene evolution of the fruits. The decrease of evolution from the maximum in Hindi fruits also led to a very low rate of evolution at day 35, and as with Zibdia M3 fruits, the decay curves show temperature sensitivity that parallels the temperature sensitivity of the maximum rate of evolution.

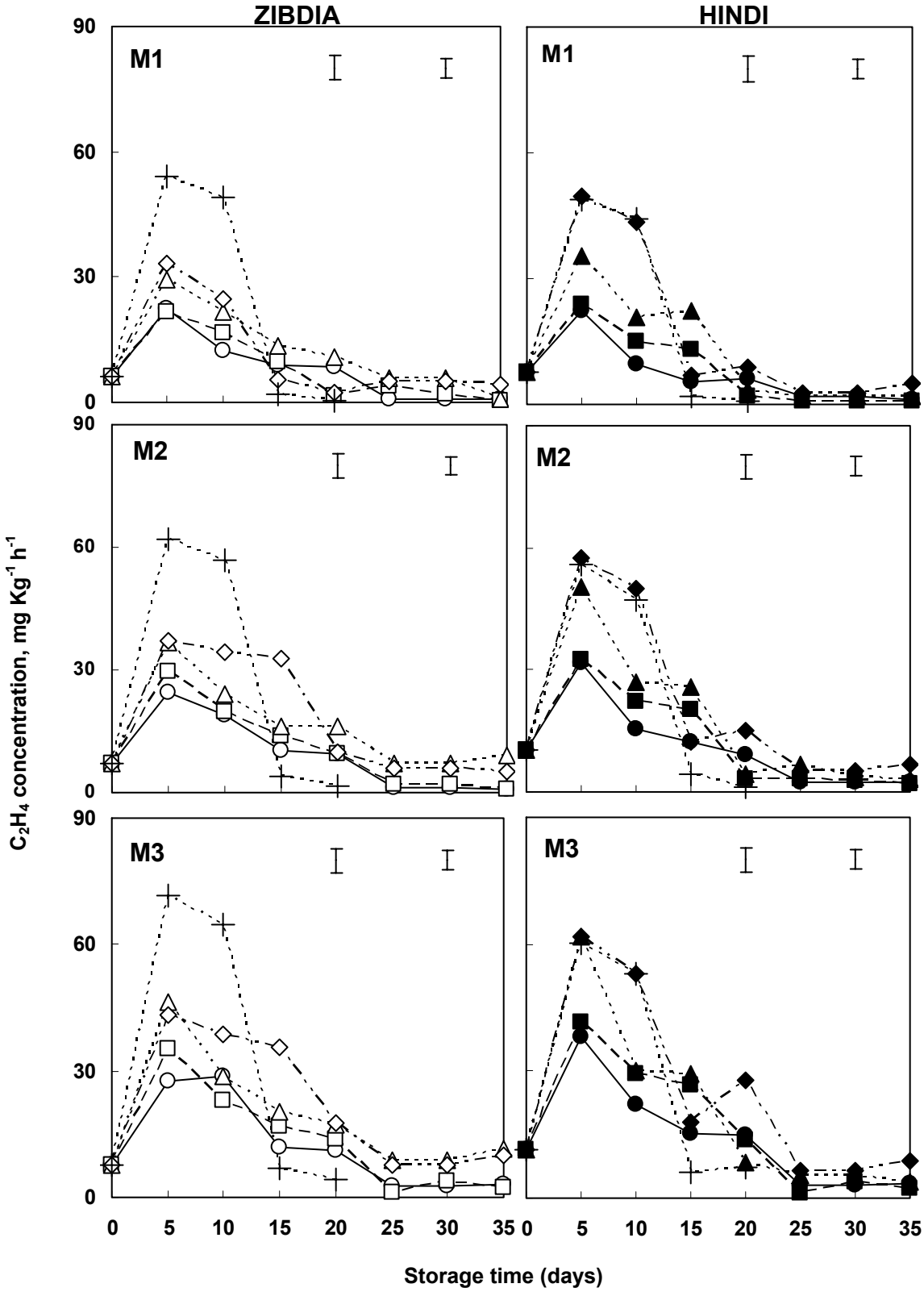

Figure1. Ethylene production versus storage time of two mango varieties Zibdia and Hindi harvested at different maturity stages: immature (M1), half-mature (M2), and full mature fruits (M3) as a function of storage time at different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■- 4, -▲- 7, -◆- 10 and -+-20^{0}C). CO_2 expressed as mean (n=3).The Vertical bars indicate L.S.D. at p=0.05 in two periods: the first period (0-20 days; all treatments present) and the second (0-35 days; when all treatments were present except for storage at 20^{o}C).

3.2. Respiration rate

Figure 2 shows the climacteric respiration rates of both mango varieties measured by CO_2 production in $mg.Kg^{-1}.h^{-1}$ plotted as a function of storage time at different storage temperatures for both mango varieties at three different maturity stages. The interaction at $p<0.001$ was significant interaction among storage time (days), temperatures, maturity and varieties. On day zero, the respiration rate was found to be slightly higher in Zibdia than Hindi fruits at all maturity stages (~4 *vs* ~8 mg kg^{-1} h^{-1}). Both varieties show a slight decrease in the respiration rate up to the fifth storage day independent of the storage temperature and/or the maturity stage. It can be seen from Figure 2 that the respiration rate shows a sudden large increase from day 5 until day 10 of storage time for both mango varieties at all maturity stages. This is followed by a sudden decrease with almost the same rate to reach similar values to that found at day 5 in all cases. This peak is very large when the fruits are stored at 20°C. Starting from day 15, the respiration rate increases slightly up to 35 days in M2 and M3 in both fruit types. For M3 fruits, the respiration rate decreases again after 30 days of storage. The variation of the respiration rate is found to be temperature dependent.

3.3. Firmness

Figure 3 shows the fruit firmness (N) of both mango varieties plotted as a function of storage time (days) at different storage temperatures for both mango varieties at three different maturity stages. The interaction at $p<0.001$ was significant among storage time (days), temperatures, maturity and varieties. The results show that Hindi fruits are initially firmer than Zibdia. Considering Hindi fruits in the different maturity stages, it is clear that the immature fruits are always more firm (160 N) than the half mature (140 N) and fully mature fruits (130 N). On the other hand, the firmness in Zibdia is approximately the same (~120 N) at all three maturity stages. Fruit firmness decreases progressively during the storage period up to the end of the experiment. Two different rates of decrease in fruit firmness can be seen at 20°C in Zibdia fruits. In M1 Zibdia fruits, a fast decrease was seen up to day 10 of storage followed by a slight decrease up to day 20, where it reached its lowest level. M2 fruits showed a modest decrease in the fruit firmness in the first 15 days followed by larg drop to a low level up to the day 20. M3 firmness decreased with a high constant rate to its lowest level at day 20. For Hindi fruits stored at the same temperature (20°C), M1 and M2 showed decreasing fruit firmness at a more or less constant rate up to day 20. M3 Hindi fruits reached their lowest firmness value after 10 days of storage. No significant differences can be seen between and in them M1 and M2 Zibdia fruits stored at 2-10°C during the first

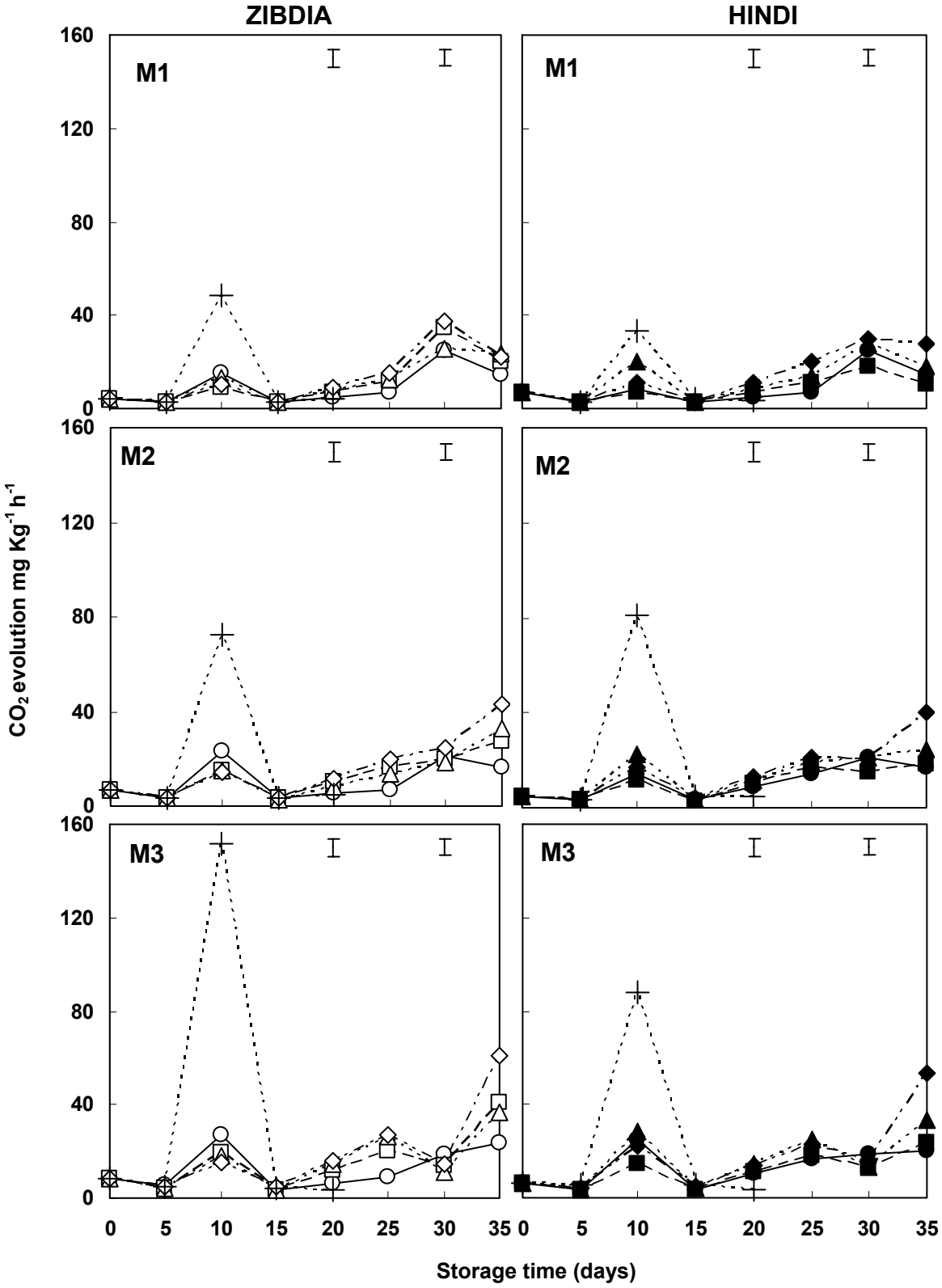

Figure 2. Carbon dioxide rate versus storage time of two mango varieties Zibdia and Hindi harvested at different maturity stages: immature (M1), half-mature (M2), and full mature fruits (M3) as a function of storage time at different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■- 4, -▲- 7, -◆- 10 and -+-20^{0}C). CO_2 expressed as mean (n=3).The Vertical bars indicate L.S.D. at p=0.05 in two periods: the first period (0-20 days; all treatments present) and the second (0-35 days; when all treatments were present except for storage at 20^{o}C).

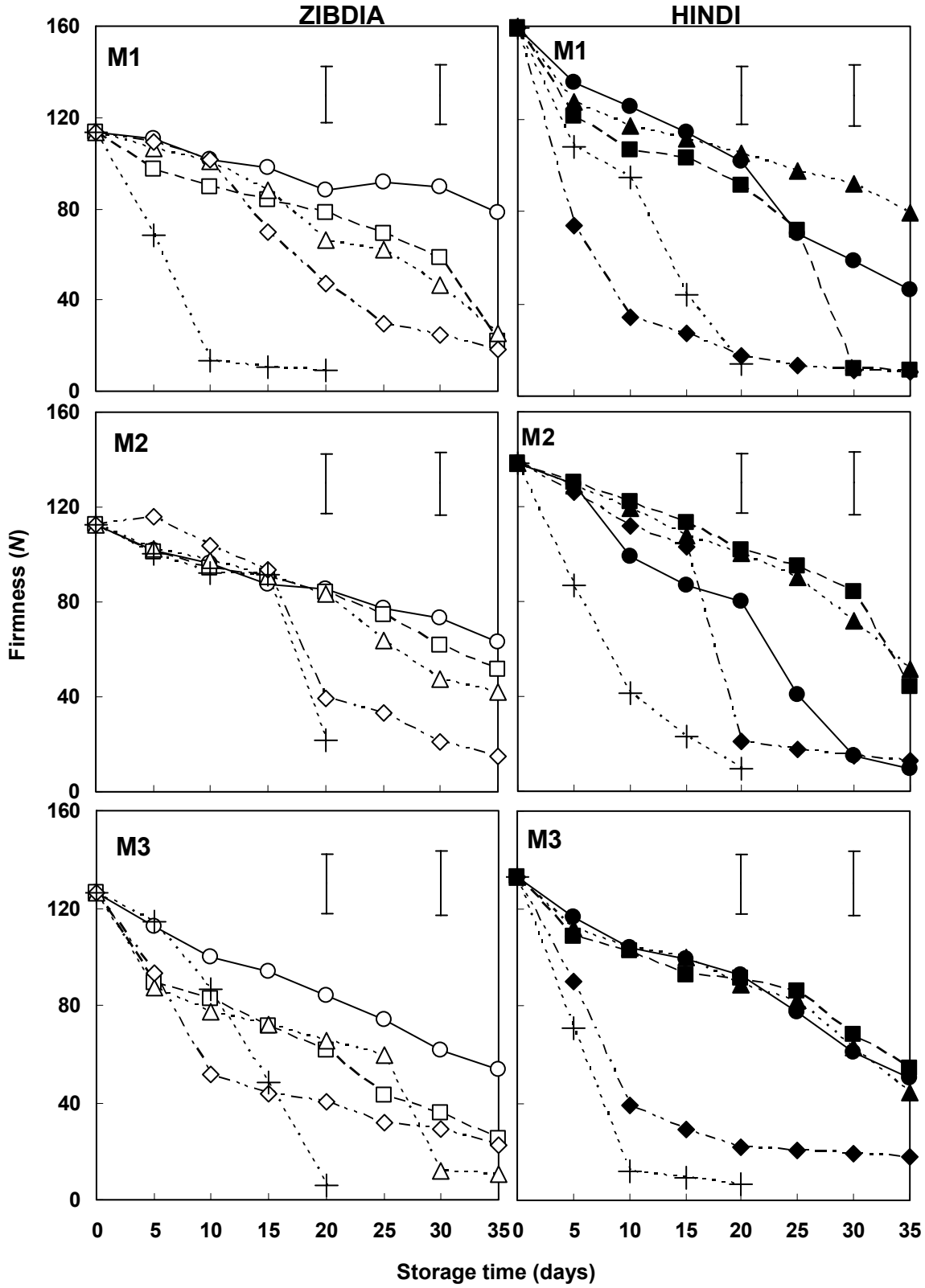

Figure 3. Firmness versus storage time of two mango varieties Zibdia and Hindi harvested at different maturity stages: immature (M1), half-mature (M2), and full mature fruits (M3) as a function of storage time at different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■- 4, -▲- 7, -◆- 10 and -+- 20^{0}C). Firmness expressed as mean (n=3).The Vertical bars indicate L.S.D. at p=0.05 in two periods: the first period (0-20 days; all treatments present) and the second (0-35 days; when all treatments were present except for storage at 20^{o}C).

10 days of storage time. The firmness of Zibdia fruits is inversely proportional to the storage temperature. The effect is more pronounced in M1 and M2 fruits in comparison to M3 fruits, and especially in fruits stored at 10^{o}C. The same behavior was also found for Hindi fruits where, at 10^{o}C, a more significant decrease started from day 15 in M1 and M2 and day 10 in M3.

2.4. Electrolyte Leakage

Changes in electrolyte leakage was described in Chapter 3 (Figure 4)

3.5. Lipid peroxidation measured as the formation of MDA equivalents

Changes in lipid peroxidation of cell membranes, caused by low storage temperature, give rise to products such as malondialdehyde (MDA) in mango fruits as described in chapter 3 (Figure 5).

4. Discussion

4.1. Ethylene production (C_2H_4)

Ethylene is a simple molecule which has profound effects on many aspects of the growth and development of plants. Plant tissues of all ages can be induced to produce ethylene in response to various challenges, and plant tissues will respond to ethylene in a range of ways. The evolution of ethylene and the response of tissues to applied ethylene are, however, under developmental control: they are to various degrees dependent upon tissue type and age. So, ethylene synthesis occurs at specific times in the developmental trajectory of certain tissues, for example during fruit ripening, leaf and flower senescence and abscission. A wide range of stresses will also provoke ethylene production, for example mechanical trauma such as bruising and cutting, temperature stresses, and chemical stress (Heun-Hong and Kenneth, 2000).

During storage, mango fruits show an initial maxima in ethylene production after day 5 of storage at all maturity stages of both varieties. This result is consistent with previous studies. The increase in C_2H_4 production rate at the day 5 may be due to an increased reaction rate of *s*-adenosyl-methionine (SAM) to 1-cyclopropane-1-carboxylic acid (ACC), which is an immediate precursor of ethylene, a reaction catalyzed by ACC synthase and ethylene forming enzymes, as recently reviewed by Baldwin (2004). Thereafter, the ethylene production rates decline gradually up to day 15 of storage in all maturity stages of both mango varieties. Storage temperature appears to be another determining factor for the formation of C_2H_4, and possibly the activity of ACC synthase.

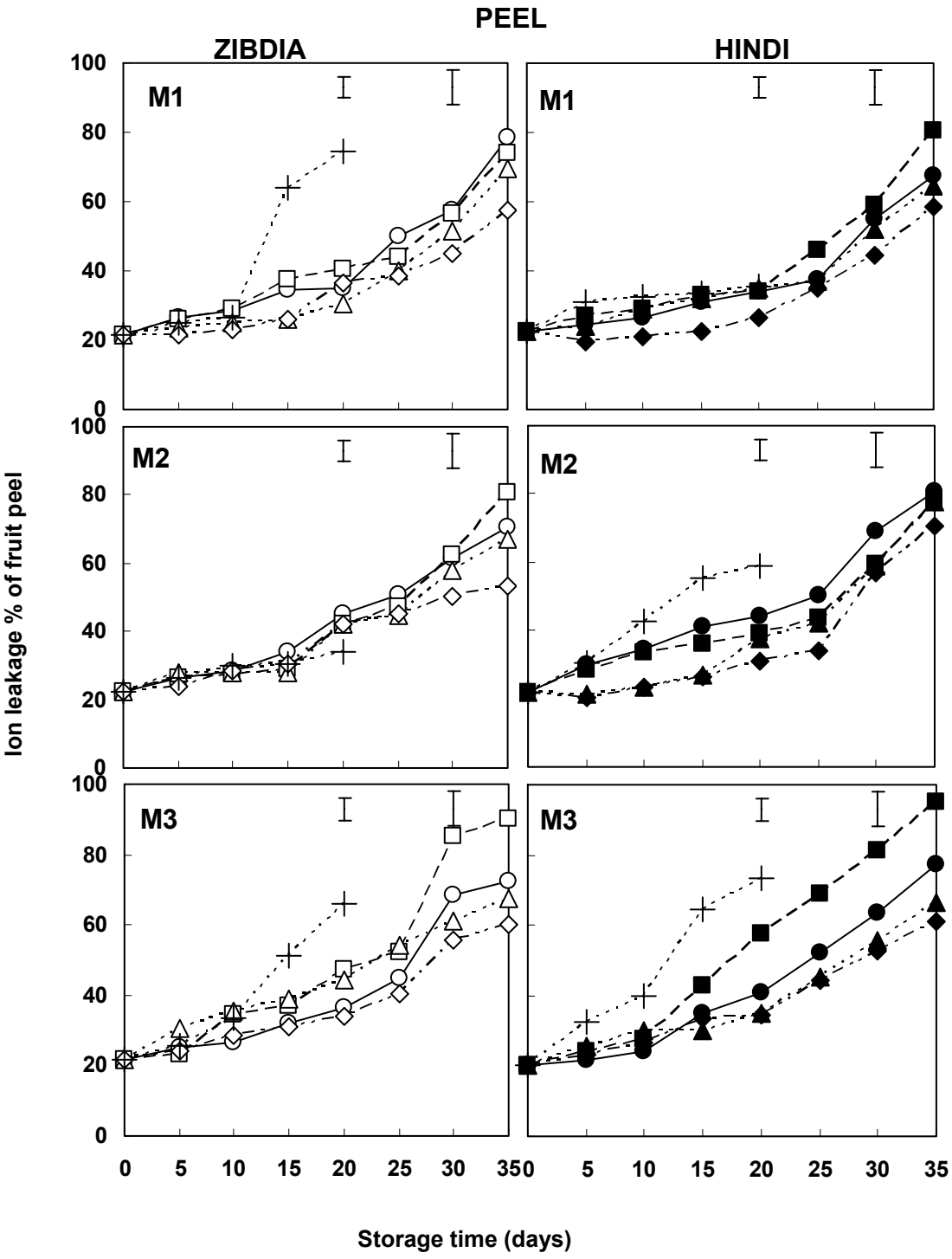

Figure 4a. Ion leakage percentage of fruits peel versus storage time of two mango varieties Zibdia and Hindi harvested at different maturity stages: immature (M1), half-mature (M2), and full mature fruits (M3) as a function of storage time at different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -O- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■- 4, -▲- 7, -◆- 10 and -+-20^{0}C). Ion leakage expressed as mean (n=3).The Vertical bars indicate L.S.D. at p=0.05 in two periods: the first period (0-20 days; all treatments present) and the second (0-35 days; when all treatments were present except for storage at 20^{o}C).

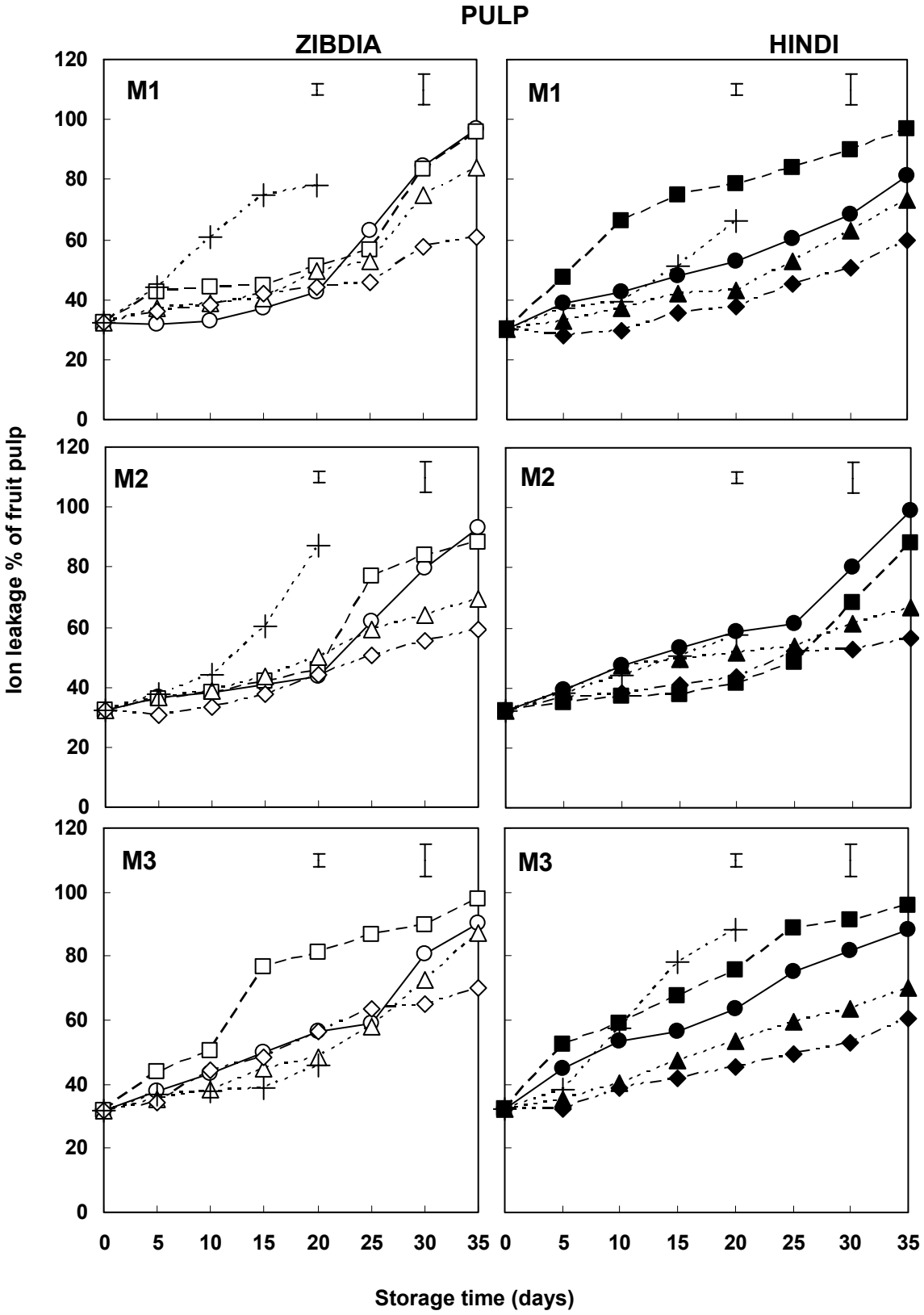

Figure 4b. Ion leakage percentage of fruits pulp versus storage time of two mango varieties Zibdia and Hindi harvested at different maturity stages: immature (M1), half-mature (M2), and full mature fruits (M3) as a function of storage time at different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■- 4, -▲- 7, -◆- 10 and -+-20^{0}C). Ion leakage expressed as mean (n=3).The Vertical bars indicate L.S.D. at p=0.05 in two periods: the first period (0-20 days; all treatments present) and the second (0-35 days; when all treatments were present except for storage at 20^{o}C).

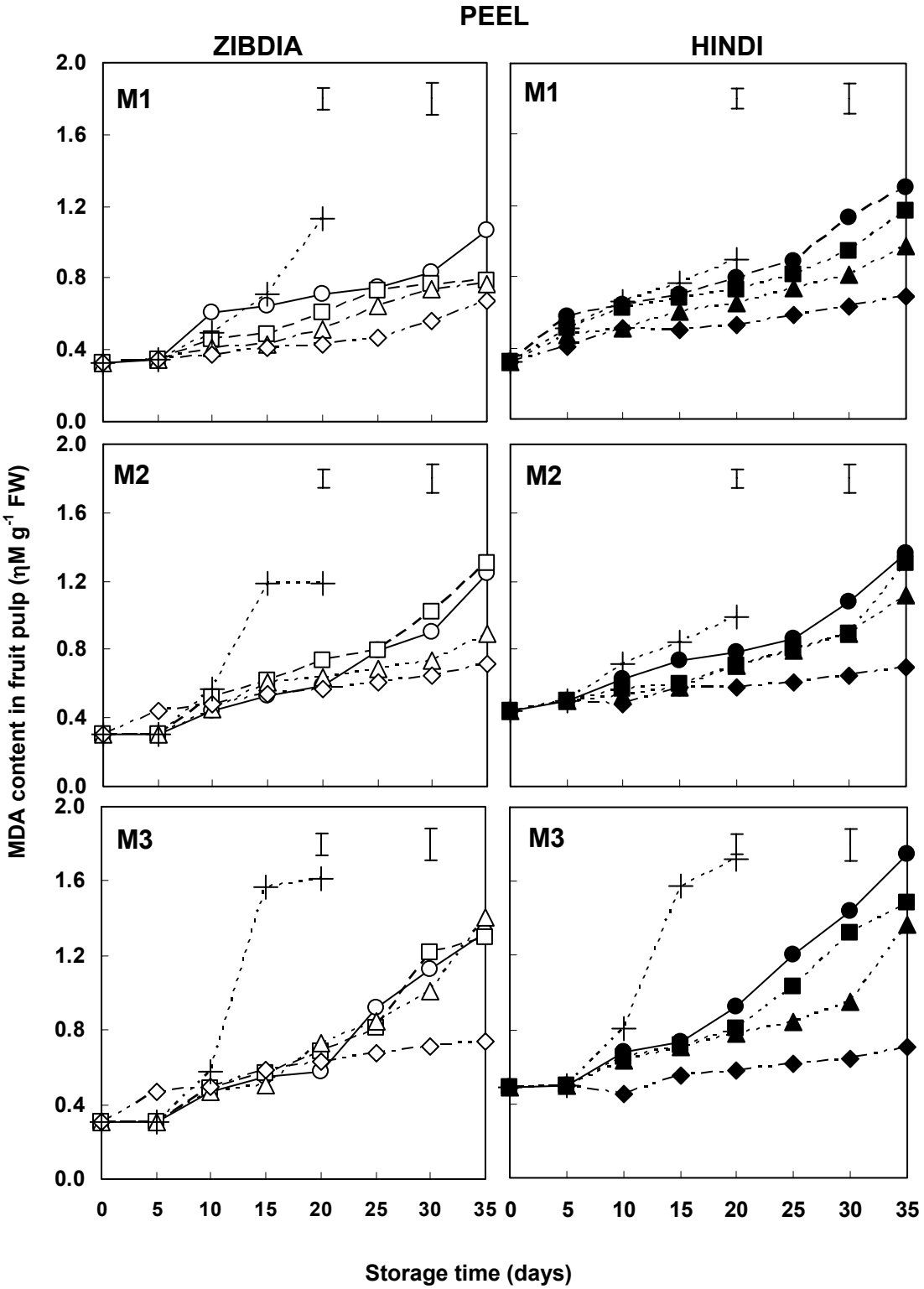

Figure 5a. Malondialdehyde (MDA) content of Zibdia (A) and Hindi (B) cultivars harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured of fruit peel stored at versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: - ● - 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). MDA content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

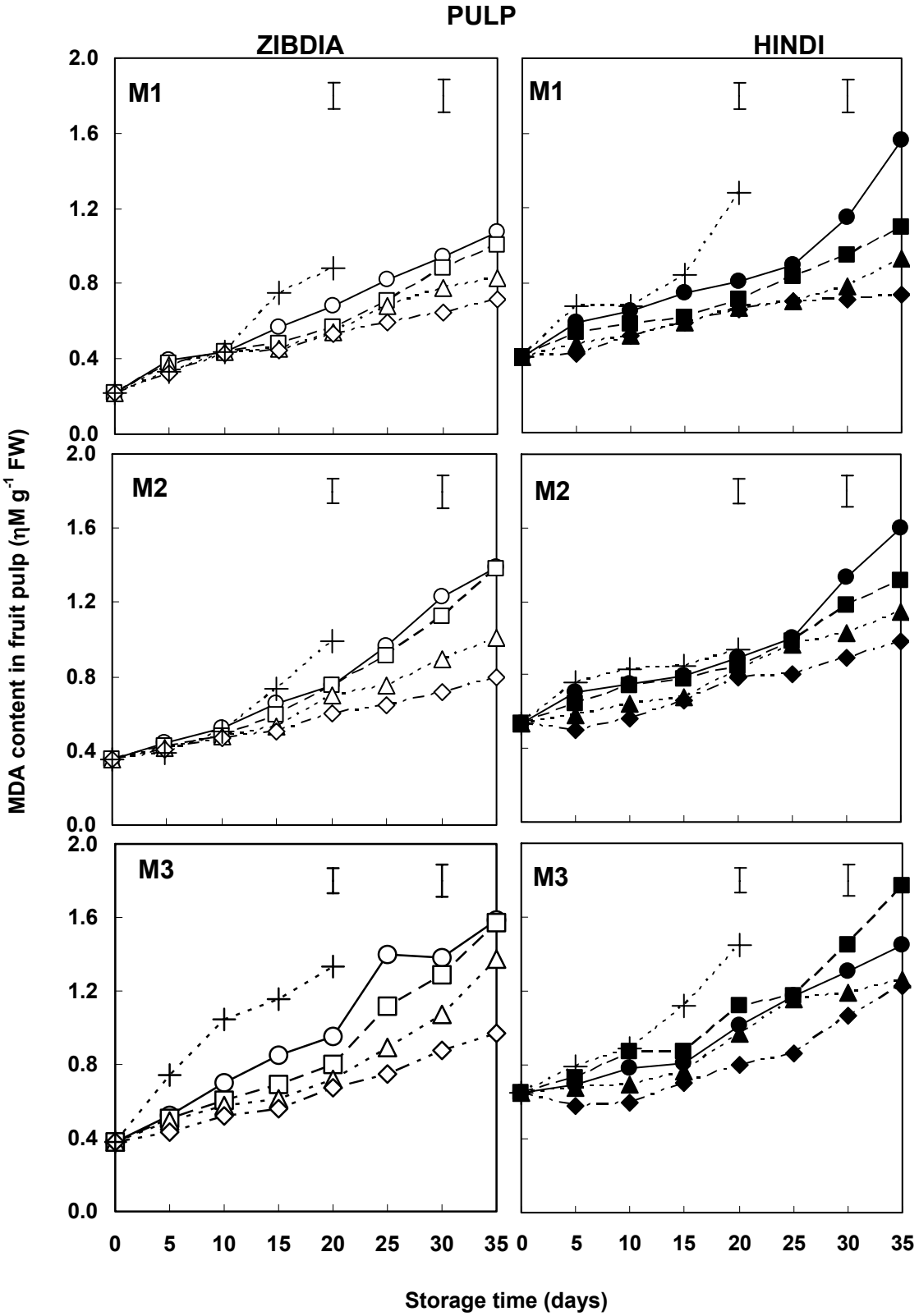

Figure 5b. Malondialdehyde (MDA) content of Zibdia and Hindi varieties harvested in different maturity stages: immature (M1), half-mature (M2) and full mature fruits (M3), measured of fruit pulp stored versus storage time at 5 different storage temperatures. Symbols represent the storage temperatures are (Zibdia: -○- 2, -□- 4, -△- 7, -◇- 10 and -+- 20^{0}C) and (Hindi: -●- 2, - ■ - 4, -▲- 7, -◆- 10 and -+-20^{0}C). MDA content expressed as mean (n=3). The vertical bars indicated to L.S.D. at p=0.05 for two period (0- up to 20 days) when all treatments were present and the second is from (0- up to 35 days) for all treatments except for storage at 20^{o}C.

The activity of ACC synthase is directly proportional to the storage temperature according to Kader (2002). This is shown for both varieties at day 5, and especially for Zibdia (Figure 1). The near zero level of ethylene production seen after 25 storage days when fruits were stored at 2 and 4^{o}C has been attributed to a lower activity of ACC synthase at these temperatures and storage times

A difference in the ethylene production rates among the different maturity stages was also observed: that M3 fruits produce ethylene at a higher rate compared to M1 and M2 fruits. The same result was reported by Kader (2002) and Majeed and Jeffery (2002). Since all the maturity stages show the same initial ethylene production rate, which thereafter changes according to the storage temperature and the maturity stage, it can be suggested that the activity of ACC synthase enzymes in forming ethylene is initiated only after fruit harvest (5 days).
The results above can be used to study correlations between ethylene evolution and lipid peroxidation (Figure 6). The severity of chilling injury symptoms developed after 20 days of storage were reported in chapters 3 and 4. The development of oxidative damage in relation to ethylene differs between the peel and pulp. Ethylene evolution reaches a maximum after 5 days of storage, and for the fruits stored at 20°C when a sharp increase in the formation of MDA equivalents develop in the peel (i.e. Zibdia M2 and M3, and Hindi M3 fruits) it does so after 5 days; so it is fair to argue that in these cases the onset of MDA equivalent formation is correlated with the C_2H_4 maximum. In the other maturity stages that do not show the sharp increase in formation of MDA equivalents in the peel samples, it is also reasonable to argue that though the fruits are producing ethylene, oxidative processes leading to membrane breakdown do not become sensitive to ethylene; if harvested in an immature phase the fruits never develop this sensitivity to C_2H_4. The change in concentration of MDA equivalents in the pulp at 20°C in relation to changes in C_2H_4 evolution differs to that of the peel, indicating that there is tissue specificity as a well as maturity dependency in the C_2H_4/oxidation relationship. The formation of MDA equivalents in the pulp does not show the dramatic increase that is evident in some of the peel samples. Rather the increase is more progressive and begins from the day 0 of storage, though some samples (M1 and M2 of Zibdia, M1 of Hindi) do show an increase of production after day 10 of storage. Under low temperature storage the formation of MDA equivalents could be influenced by both chilling and C_2H_4. Relative to the 20°C storage the rise in the concentration of MDA equivalents is slower at chilling temperatures, and is increased by decreasing temperatures.

Considering firstly processes in the peel, the increase in MDA equivalents at low temperatures for Zibdia M2 and M3 and Hindi M3 differs markedly from that at 20°C, whereas MDA equivalents formation in Hindi M1 and to a lesser extent M2 is similar to that at 20°C.

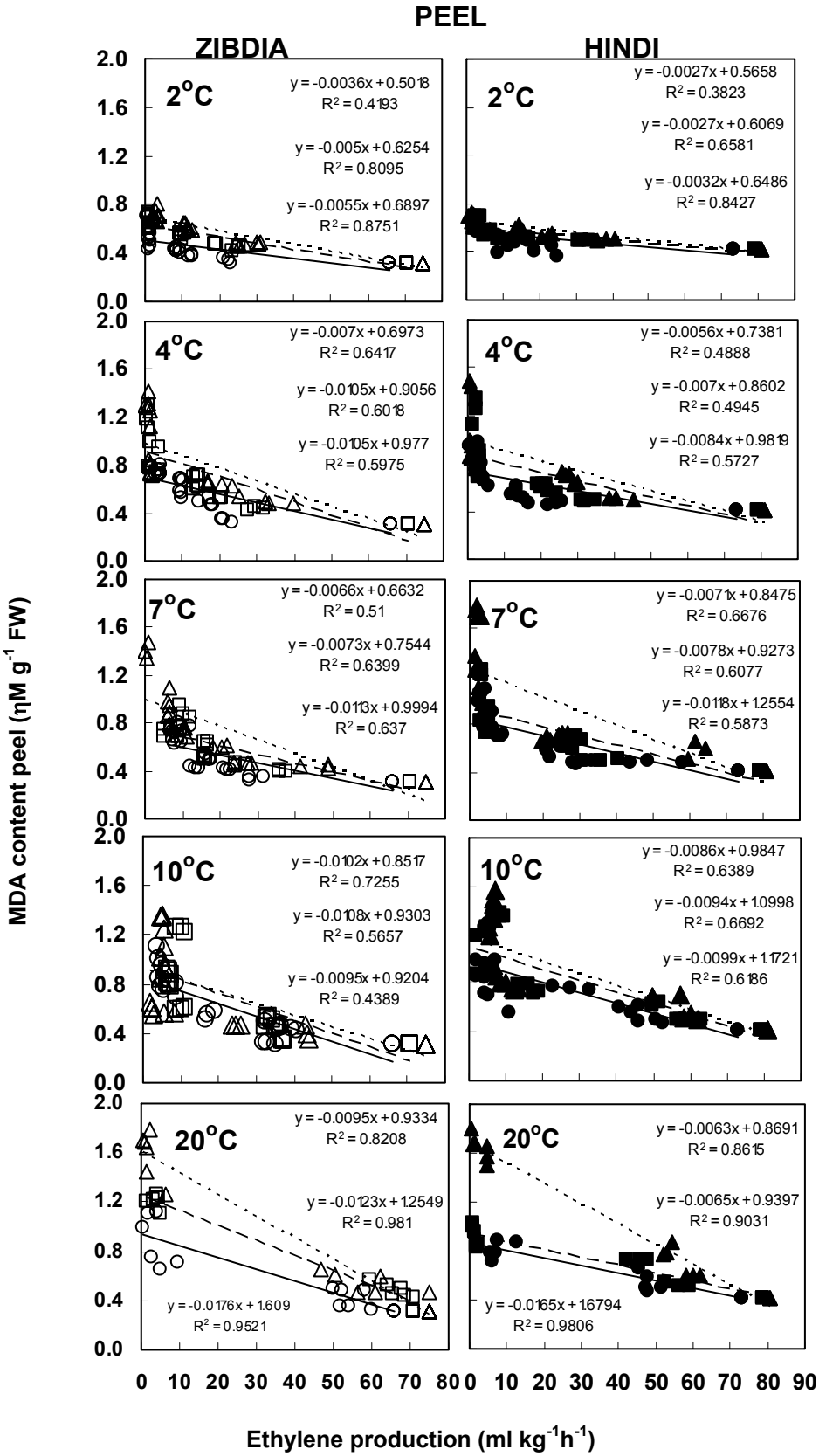

Figure 6a. The linear relationship of malondialdehyde (MDA) content of fruit peel in function of ethylene production rate on fruits of Zibdia and Hindi varieties harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days.

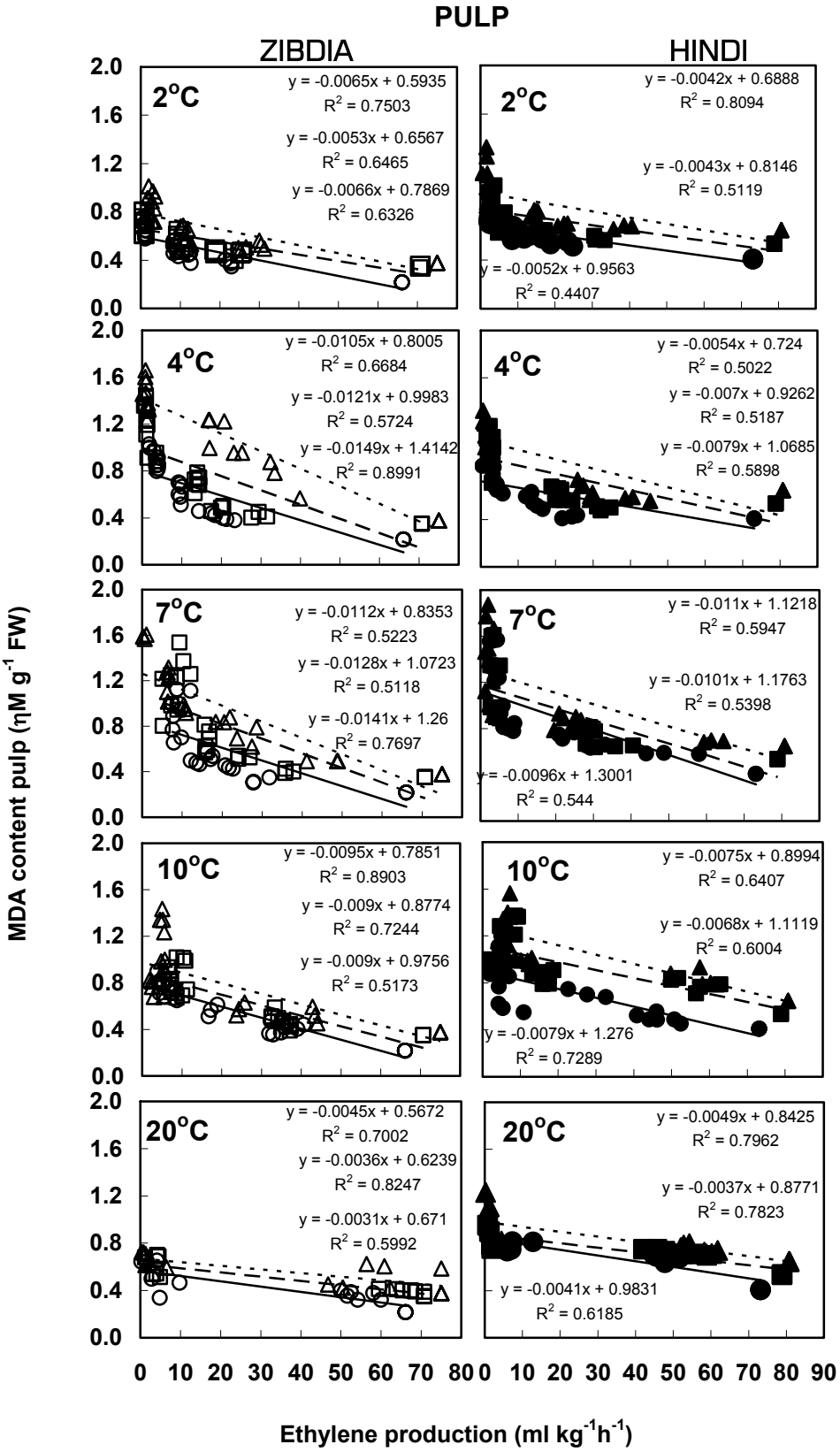

Figure 6b. The linear relationship of malondialdehyde (MDA) content of fruit pulp in function of ethylene production rate on fruits of Zibdia and Hindi varieties harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days.

These responses do not have any strong correlation with changes in C_2H_4 formation; plotting the MDA equivalent content against C_2H_4 formation (Figure 6) reveals that, overall, as C_2H_4 evolution decreases then MDA content increases, but that in detail the increase in MDA content can be seen to increase strongly when the rate of C_2H_4 evolution has fallen to low values (e.g. Figure 1, 4°C data). In the pulp under low temperature storage there is also no clear correlation between C_2H_4 formation and MDA formation. For example the large variation in C_2H_4 formation that occurs in the M3 stages of both Zibdia and Hindi is not paralleled by similar variations in MDA concentration. Nor is there for the pulp at low temperatures any evident temporal correlation between C_2H_4 formation and the increase in MDA concentration. The conclusion is therefore that though C_2H_4 may be correlated with increases in membrane oxidation in the peel at 20°C at more advanced maturity stages, there is no clear correlation at low temperatures in the peel, or for the pulp at any temperature. Note, however, that the absence of a correlation does not rule out an indirect causality. Another conclusion that can be drawn from the low temperature data is that though it is possible for C_2H_4 to be formed as an oxidized fatty acid breakdown product, the decrease of C_2H_4 evolution with increasing MDA, and the independence of C_2H_4 evolution shown when C_2H_4 evolution is low and MDA levels are increasing (Figure 5), indicates that compared to biochemical formation of C_2H_4 the non-enzymic production by membrane oxidation is insignificant.

4.2. Respiration rate (CO_2)

Respiration is the process by which stored organic materials (Carbohydrates, proteins and fats) are broken down into simple products such as carbon dioxide and water vapour and accompanied by evolution of energy (Kader, 2002; Noodén and Leopold, 1988). The loss of stored food reserves in the fruit through respiration means the hastening of senescence. As the reserves that provide energy to maintain living status are exhausted, food value for the consumer is reduced through loss of flavor quality and loss of salable dry weight. The rate of deterioration of harvest commodities is generally proportion to the respiration rate. Horticultural crops can be classified according to their respiration rates and mango fruits are classified as moderate respiration (Kader, 2002). Several studies have reported that ethylene activates respiratory enzymes in different processes in different fruits. For example, ethylene treatment increases the activity of malic enzymes in climacteric fruits (pome fruits) and the respiration of avocado and banana in the pre-climacteric stage, as reviewed by Noodén and Leopold (1988). Mango fruits undergo an initial climacteric respiration, reaching a maximum at day

10 of storage at all maturity stages (Figure 2). This is five days later than the maximum of C_2H_4 evolution, and this result differs from previous reports.
The increased respiration rates of Zibdia and Hindi mango varieties occurred at day 10 of this experiment (after 5 days of the ethylene peak). A similar pattern was described previously for the 'Kent' mango variety, but a reversed pattern was observed in the 'Haden' variety (Trinindad et al., 1997). The variation of the order of respiration and ethylene production after harvesting indicates that ethylene and respiration production depend on mango varieties which (McCollum et al., 1993). It is suggested that in our case, ethylene production is an important factor in the activation of respiratory enzymes. The observed climacteric burst at day 10 of storage may be caused by enhancement of the phosphorylation process, increasing concentration of ATP as an essential energy molecule for the chemical reactions of respiration (Noodén and Leopold, 1988). This peek is very temperature sensitive (Figure 2). After the climacteric peak, respiration rate decreased rapidly to very low levels after 15 days of storage. This low level is maintained up to 35 days but with a slight increase with increasing storage time in all maturity stages of both mango varieties. These increases of respiration were found towards the end of the experiment (days 20-35). This is in accordance to the previous results by Dinora et al. (1997). Increased CO_2 production by increasing maturity of fruit was also observed in 'Tommy Atkins' by Majeed and Jeffery (2002). We found a positive relationship between MDA and respiration in both mango varieties (Figure 6). However, the correlation was generally above 50% in the cold stored mango whereas it and was much less in the case of mango peel stored at 20^{o}C for both varieties. The relation was more significant when storage temperature was lowered (2^{o}C to 10^{o}C) compared with 20^{o}C for fruit pulp. These patterns shown suggest that respiration may affect peroxidation of lipid cell membrane indirectly, by forming AOS during respiration processes (Meir, 1986). It would be also be that as the rise of MDA indicates increase membrane damage, some uncoupling of respiration may have occurred. Enzymes and non-enzyme antioxidants declined until the end of the experiment as described in chapters 4 and 5.

4.3. Firmness

Firmness is one of the most important product attributes determining product acceptability to the consumer. As an attribute, firmness depends on many properties of the tissue: water content, the nature of the cell wall and turgor are clearly important sources of firmness, but the cell contents can also play a part. Changes in firmness should, in principle, be traceable to alterations in the various tissue components or properties that combine to create the firmness attribute, and

PEEL

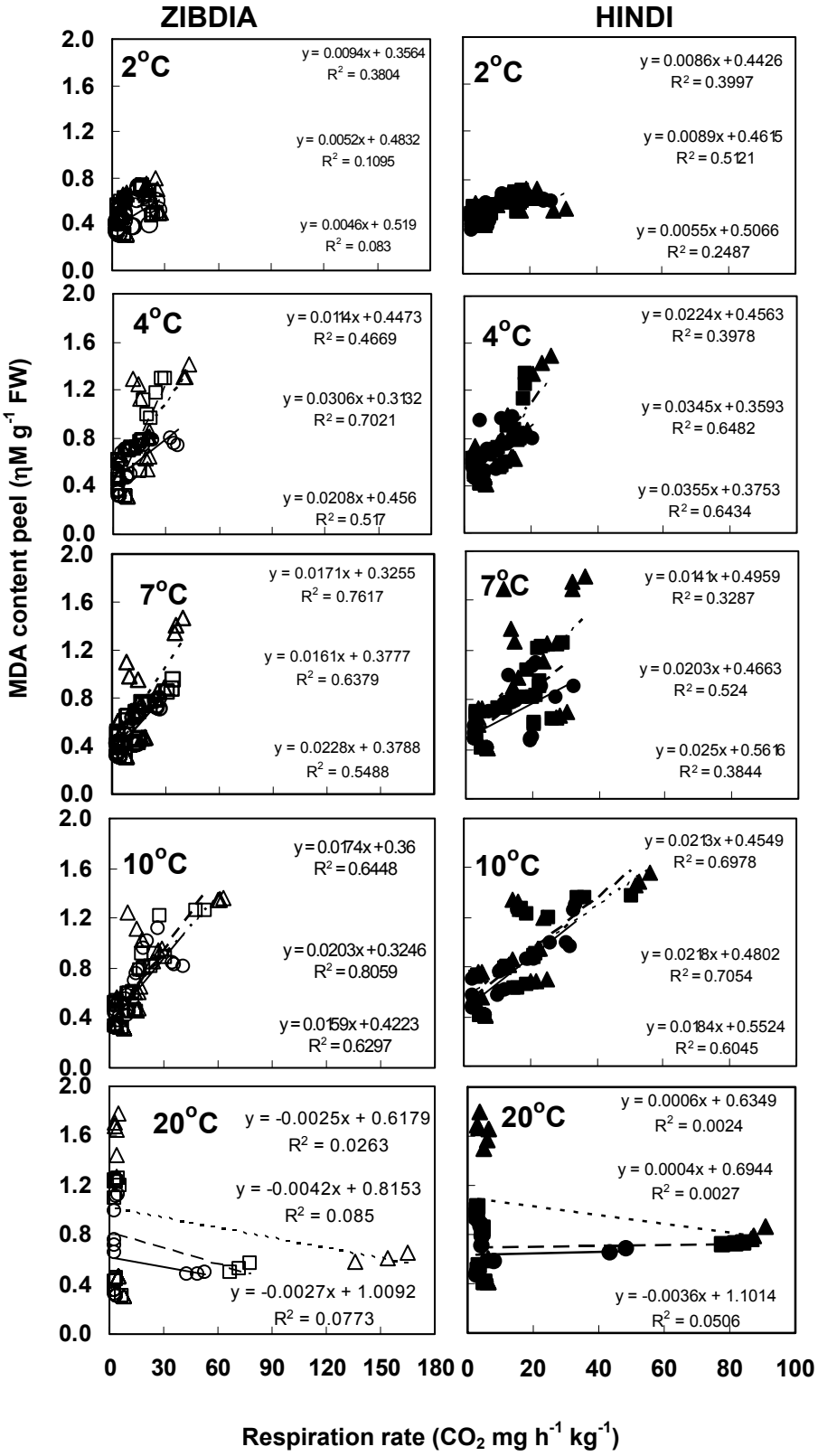

Figure 6a. The linear relationship of malondialdehyde (MDA) content of fruit peel in function of respiration rate of fruits of Zibdia and Hindi varieties harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days.

PULP

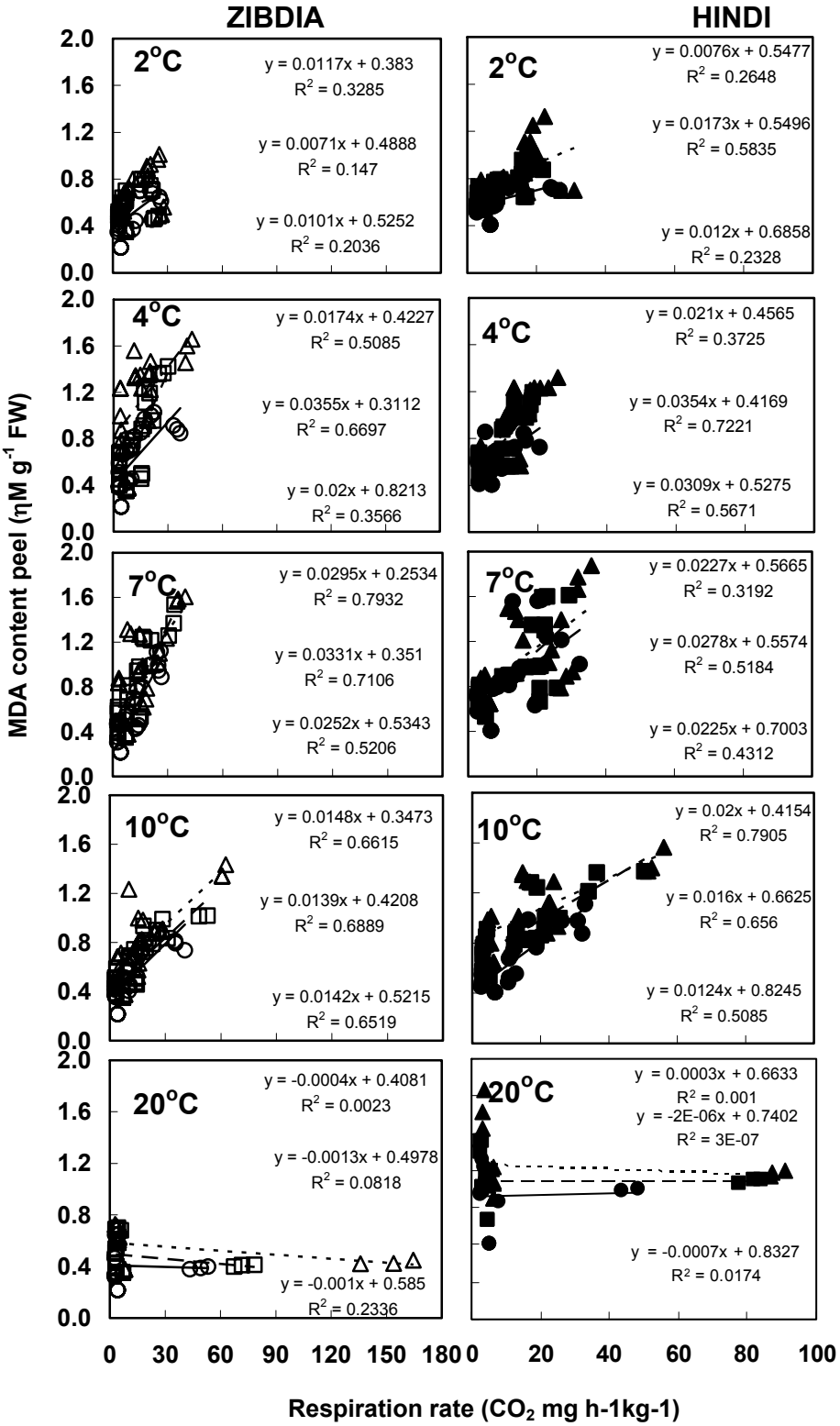

Figure 6b. The linear relationship of malondialdehyde (MDA) content of fruit pulp in function of respiration rate of fruits of Zibdia and Hindi varieties harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days.

storage conditions should be chosen to minimize alterations in these components and properties (Sams, 1999).

The mechanical nature of firmness has lead to attention being directed towards cell wall changes. (Ketsa et al., 1999b; Watada et al.,1984). The breakdown or modification of cell wall polysaccharides during the ripening of mango fruits is associated with changes in firmness, and these changes are the result of the increased activity of polygalacturonases, cellulase and β-galactosidase (Ali et al., 1995; Huber, 1983, Ketsa et al., 1999b). Based on these, an explanation could be suggested for the rapid decrease in firmness of Zibdia and Hindi fruits when they are stored at 20^{o}C for 20 days. During this period, hydrolase enzymes may act on the cell wall resulting in the rapid decrease of fruit firmness as shown in Figure 3. In connection, these chemical changes can be associated with increased C_2H_4 and respiration during storage of fruits at 20^{o}C by which the cell wall hydrolysis is enhanced. This is clear from our results that the decrease in fruits firmness after day 5 of storage at 20^{O}C (Saltveit, 1989). It is clear that the metabolic interaction between ethylene synthesis, respiration and decrease in fruit firmness may be attributed to increases of AOS levels during these processes. The increase in oxidation is coupled with a decline in antioxidant enzyme activity (see chapter 5), which eliminate AOS formation during the ripening processes (Aharoni et al., 2002). These responses may be stimulated by AOS formation during cell membrane peroxidation, further accelerating the ripening and senescence processes (Hodges, 2003).

Decreasing firmness is closely related to increasing amounts of MDA in the peel and pulp of both fruits (Figure 5). This relationship is consistent in both cultivars and across the three maturity stages under conditions of low temperature storage. At 20°C the relationship between MDA equivalents and firmness remains to only a limited degree for the peel, but has disappeared for the pulp. This suggests a role for oxidative stress in modulating the loss of firmness under low temperature conditions, but to a much lesser degree under 20°C storage. More broadly, it may the under low temperature storage the ripening and senescence processes are modulated by oxidation. By accelerating the ripening process AOS can lead to firmness changes via increases in enzymatic cell wall hydrolysis. In addition, however, AOS can directly break down the cell wall (Hodges 2003). They may also reduce firmness by damaging the cell membrane, and reducing turgor formation: turgor requires both a cell wall and a cell membrane, and though much attention is directed to the role of the cell wall, the membrane may also play a part. Indeed, one interpretation of the relationship between firmness and the accumulation of MDA (Figure 7) equivalents is that the loss of firmness is due to oxidative membrane damage, rather than a more general AOS induced

senescence. Fruit firmness is correlated not only with the accumulation of MDA equivalents, but also with increasing ion leakage (Figure 8) from both peel and pulp samples for both cultivars and over all temperatures and maturity stages. Membrane leakiness would be expected to be associated with a reduction of turgor and firmness, and oxidative damage to the membrane will increase leakiness. However our data do not exclude the possibility that membrane leakiness is a consequence of cell wall breakdown and not a cause of loss of firmness.

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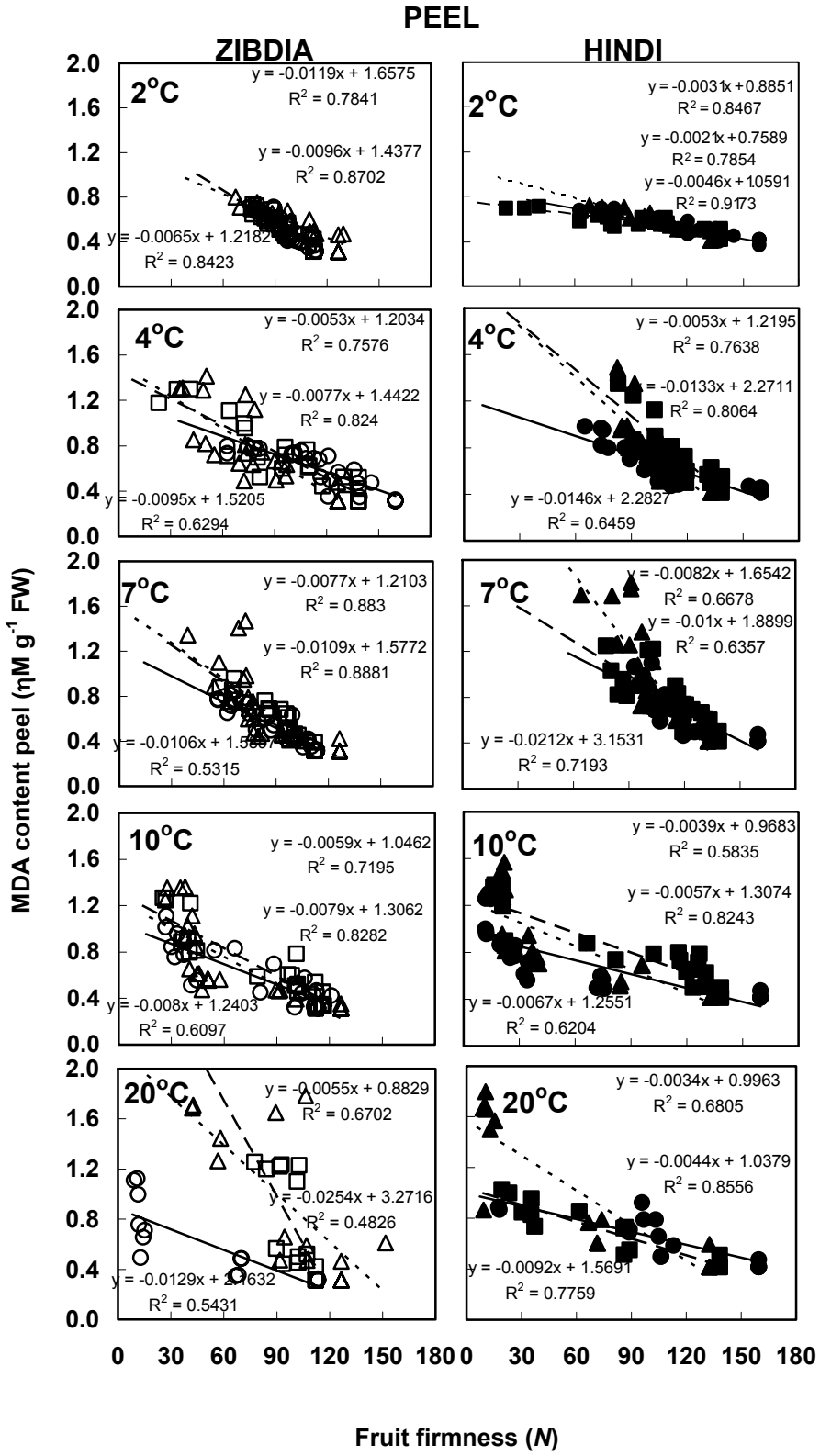

Figure 7a. The linear relationship of malondialdehyde (MDA) content of fruit peel in function of firmness of fruits of Zibdia and Hindi varieties harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days.

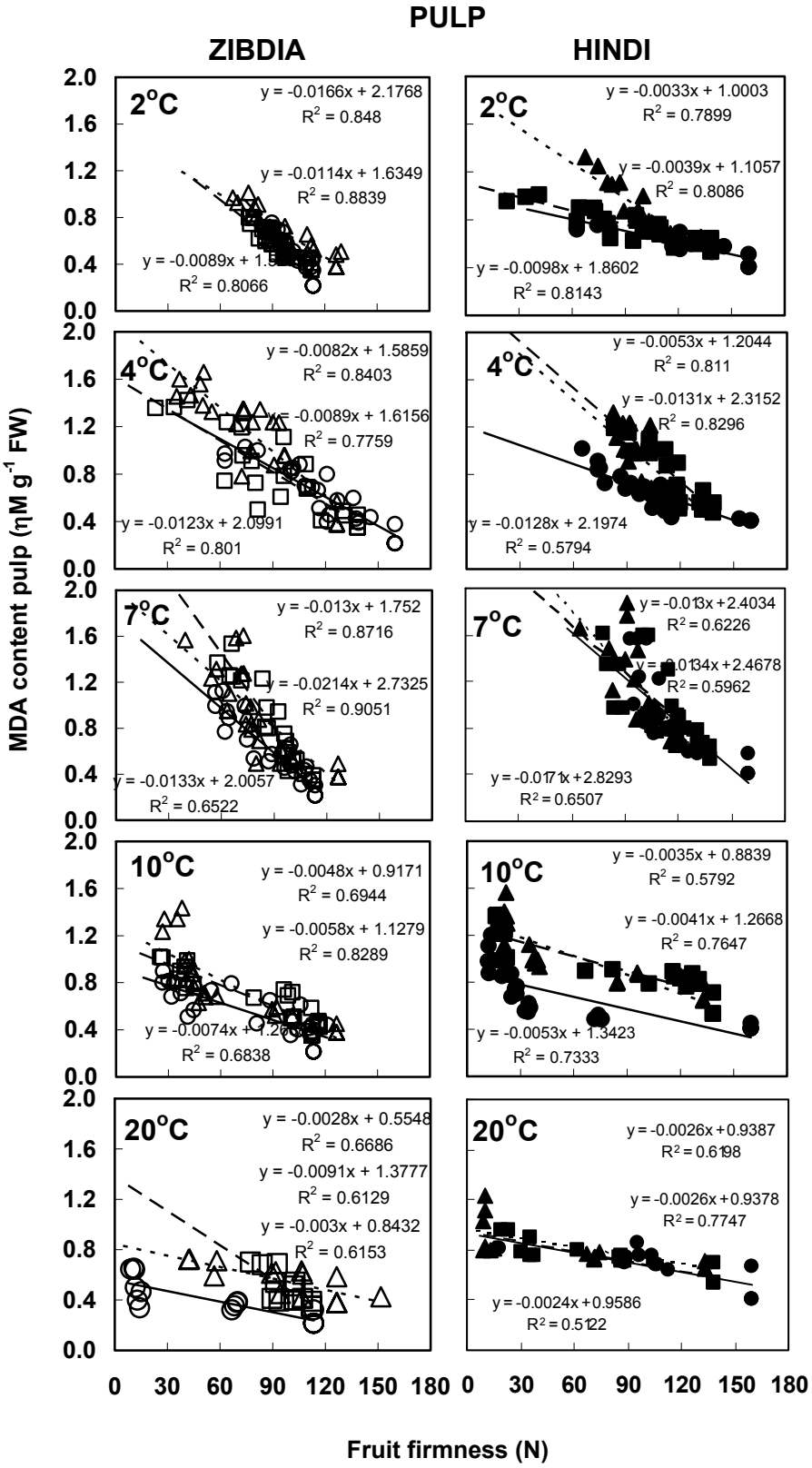

Figure 7b. The linear relationship of malondialdehyde (MDA) content of fruit pulp in function of firmness of fruits of Zibdia and Hindi varieties harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days.

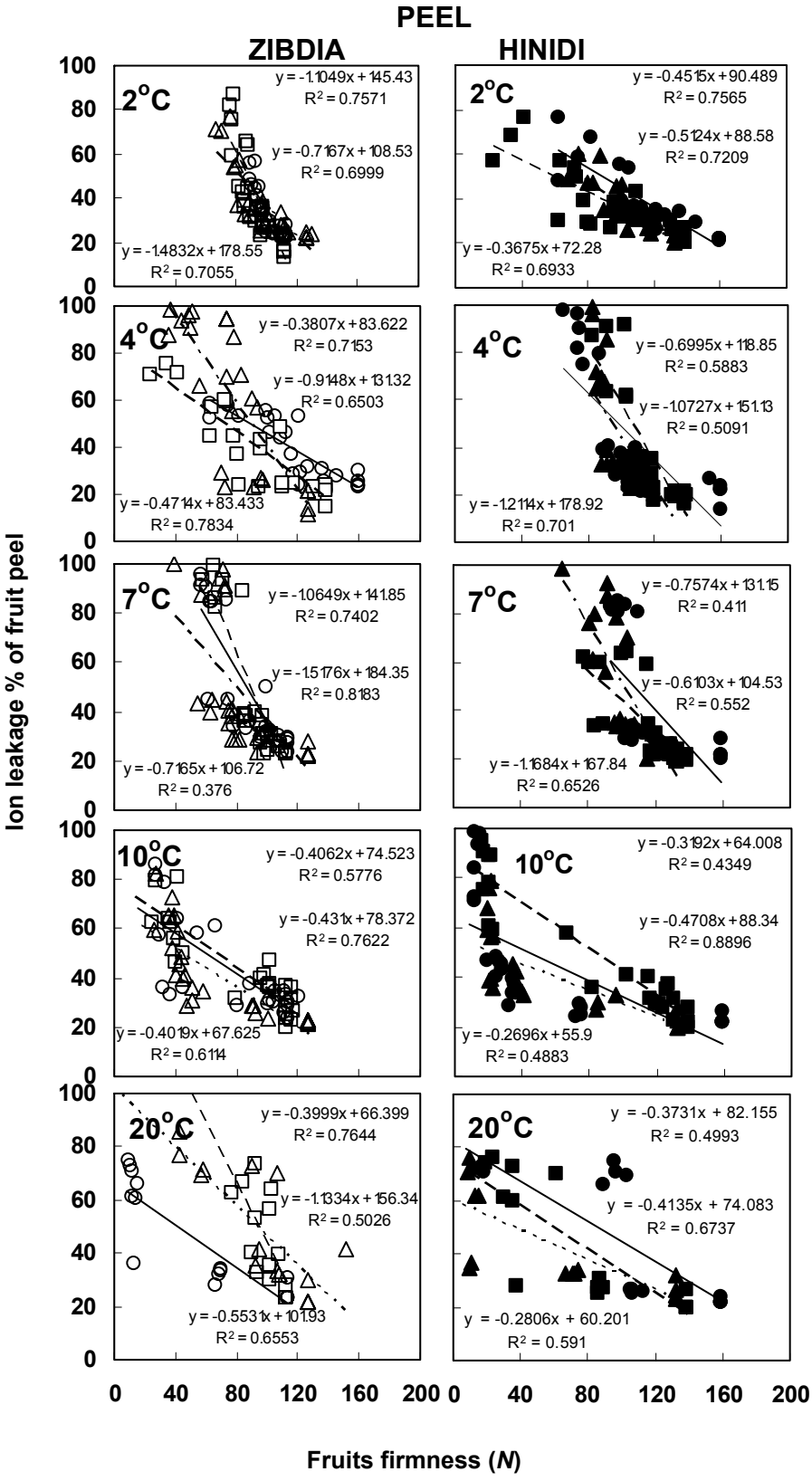

Figure 8a. The linear relationship of malondialdehyde (MDA) content of fruit peel in function of fruits firmness of Zibdia and Hindi varieties harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days.

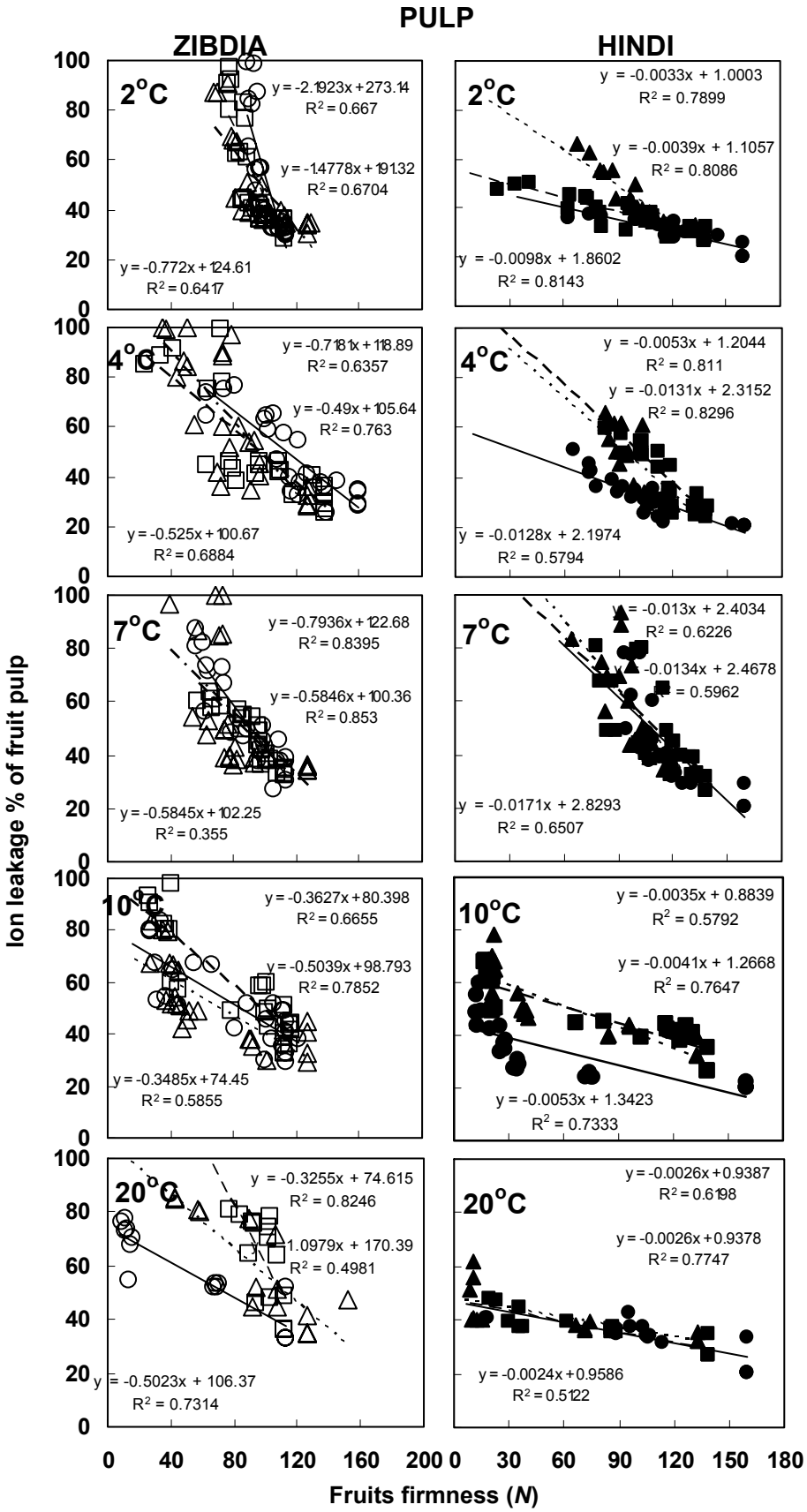

Figure 8b. Presents the linear relationship of malondialdehyde (MDA) content of fruit pulps in function of firmness of fruits of Zibdia and Hindi varieties harvested in different maturity stages (Zibdia: -○- M1, -□- M2 and -△- M3) and (Hindi: - ● - M1, - ■ - M2, and -▲- M3), which stored at different storage temperatures (2, 4, 7, 10 and 20°C), over 35 days.

Chapter 7

Final comments

Perhaps unsurprisingly, the results presented in this thesis indicate that Mango fruits are indeed chilling sensitive, which is entirely in accordance with the known physiology of this product. The detail offered in this study, however, reveals many new features of the response of these fruits to chilling. One of the most surprising was the extent to which organized metabolism could continue in these tropical products even at 2°C. Both protein and metabolite synthesis were shown to be active at this temperature, which confounds the usual impression that tropical plants products at such temperatures are at best essentially metabolically inactive. The greater damage that often occurred at 4°C compared to 2°C was also unexpected. This implies that chilling injury, though dependent on low temperatures, also has an activation energy and that it therefore also has a positive temperature dependence. Coupled with the previous comments about continued metabolic activity, the possibility remains that chilling injury in these fruits has a metabolic component.

As far the detailed properties of chilling sensitivity go, it is clear that storage at 10°C or possibly even 7°C is feasible, at least as far the physiological status of the fruits is concerned. At 10°C temperature the development of chilling related injuries was least severe yet there was some low temperature induced suppression fruit quality due to ripening. However suitability for 10°C is cultivar dependent. For example, the progress of fruit softening in both Hindi and Zibdia depended on both cultivar and maturity stage; in Zibdia the M1 fruits showed slower softening at 10°C compared to 20°C, whereas in Hindi 10°C storage did not retard softening, except in the M2 fruits. In any case 10°C storage gave at most 15 days before softening commenced. However, though in Hindi, though not greatly retarded by storage at 10°C, was markedly retarded by storage at lower temperatures (eg 7°C). These lower temperatures, however, though retarding the loss of fruit firmness, accelerated the development of chilling induced injuries. At 7°C storage, the CI-index began to increase from 1 (the value representing no damage) after the 20th day of measurement. Ion leakage and equivalents also increased more at 7°C than 10°C, though the difference was only slight. The ascorbic acid and tocopherol content of the pulp also only decreased slightly more at 7°C storage compared to 10°C, though the rise in carotene levels was conspicuously lower at 7°C. So, limited low temperature storage is possible, especially if attention is paid to cultivar selection, and certainly storage at 10 - 7°C, which seems realistic in physiological terms, is better than storage at 20°C

With regards to storage the future use of the fruit is a major that should guide the choice of storage conditions and duration of storage. The levels of ascorbic acid and tocopherol were decreasing from the first day of measurement, and in the case of ascorbic acid, levels could decrease to nearly zero. In the case

of these vitamins (C and E) any use of for which a high vitamin content was important would require immediate processing. Carotene, on the other hand, increased during the storage period, so for high carotenoid levels, storage is benefical, though processing or use would need to be synchronized with the maximum of carotenoid production, as carotenoid level decrease rapidly oncd they have reached a maximum. Genetic and tissue variability in the initial quantities and rate of change of content of components (eg vitamin content and change) and properties (firmness) is apparent even in this limited study of two cultivars. This suggests that more purposeful breeding of cultivars for more clearly defined purposes (eg storage, high carotene content, high vitamin C content) would be a possible means by which the usefulness and quality of mango fruits could be improved.

With respect to the origins of chilling injury in a shift in the balance between oxidative processes and anti-oxidative mechanisms, the number of different properties measured allows more insight to be gained as to the mechanisms that seem to be important in causing or preventing oxidative injury to the tissues of mango fruits. A major problem with the interpretation of the type of data available is its intrinsic complexity. Oxidative and anti-oxidative processes exist in a network rather than in linear sequences of reactions or events. Such networks are fundamentally more difficult to analyse in terms of limiting steps or bottlenecks. We have not had sufficient control over the mango system to allow it to be manipulated in a way that would allow a more sensitive analysis of flux control or causality. Nonetheless, some relationships were conspicuously strong, which suggests that would be worth exploring in more detail. The amounts of both ascorbic acid and tocopherol seem to correlate well and inversely with various indices of damage; MDA formation and CI-index. Especially with MDA accumulation and ion leakage the relationships were consistent across the two varieties. Ion leakage and MDA accumulation were also closely related; this is a particularly intriguing correlation because of the debate concerning the relative roles of membrane dysfunction and oxidative damage as the primary cause of injury. These data show that changes in antioxidant status are closely coupled in time to increases in lipid oxidation and ion leakage. This could be interpreted as indicating that the processes of oxidation and membrane stability changes are tightly linked and interdependent. The close relationship between ion leakage and membrane oxidation is especially important as it illustrates graphically the importance of protecting the membrane against oxidation.

The antioxidant metabolites exist in a network with antioxidant enzymes. The relative important of different enzymes in different contexts is rarely explored in depth, however the data presented here does allow something to be said about the

relative importance of different enzymes in relation to protection against different injuries. Considering ion leakage, MDA accumulation and protein carbonyl levels as indices of damage, it is clear that the intensity of damage was not correlated to SOD or GR activities. These indices of damage were better correlated with the activities of APX and CAT, though the correlations were not as homogeneous as those encountered when the concentrations of ascorbic acid or tocopherol were compared against indices of damage. They were typically more dependent on maturity stage, for example. Nonetheless, it is suggested that the activities of CAT and APX and their role in protecting against damage should be further investigated. Finally regarding the consequences of damage, it is interesting that a possible role has been identified for membrane damage and leakiness in the loss of firmness that develops during storage at low temperatures. Future research should establish the relative importance of cell wall and cell membrane changes with regards the softening of tissue during ripening and chilling injury.

The last comments concern the measurement of chilling injury. Though fluorescence has been widely used as a tool with which to measure ripening or injury, it appears that quantitatively the Fv/Fm parameter, at least as far as mango fruits under chilling conditions are concerned, does not correlate very well with other indices of damage, such as electrolyte leakage, chlorophyll content or CI-index. When measured from fruits that have been subjected to cold storage conditions, these indices correlate well with each other, so they appear to consistent indices with which to measure chilling injury. However, qualitatively the fluorescence Fv/Fm parameter correlates with other indices under chilling conditions, and the relationship is quantitative for fruits ripening at 20°C. These results suggest the need to re-examine the use of the Fv/Fm parameter as a tool for quantifying chilling injury in mango, and possibly to combine it, or even replace it, with other techniques, such as colour measurements or bioimpedance measurements.

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Summary

At present, the value and production quantity of mango fruits are increasing worldwide. Many studies emphasize how chilling injury phenomena affect the quality of tropical fruits, such as mango, during postharvest handling, transport and storage. Since mango is one of the most favored and popular fruits in the world market, and is considered to be a climacteric fruit, ripening rapidly after harvest, it is essential to study how storage affects the external and internal fruit quality. Generally, cold temperatures are used to slow metabolic processes during storage in order to extend the shelf life. Mango fruit is characterized by short shelf life at ambient conditions but it is also sensitive to low storage temperatures. Therefore, the susceptibility and sensitivity of mango fruits to low storage temperatures (ie below 12^{o}C) coupled with their perishability at non-chilling temperatures limits the handling, storage and transportation of the fruits. Because of this, the pre and post-harvest factors involved in the susceptibility of mango fruits to chilling injury must be carefully studied together to improve shelf life and quality of fruits.

The main aim of this study was to quantify and understand the effects of storage conditions on the occurrence of chilling injury to mango fruits during long-term storage at low temperatures. Critical attention has been paid to the integration of factors that might contribute to susceptibility to injury and attempting to develop a better understanding of the physiological processes involved with chilling injury in order to better predict and understand chilling injury. The focus was on the effect of storage conditions (temperature and duration of storage), on mango varieties (Zibdia and Hindi Be-Sennara), harvested at different fruit maturity stages, immature (M1), half-mature (M2) and full mature (M3), with regard to the appearance of chilling injury symptoms.

Chapter 1 presents an overview of mango as plant, crop, and economic commodity in both international and local markets.

Physiologically, the effect of low temperatures on plant cells induces physical and/or physiological disturbances, or injury, in cell membranes and cytosol Based upon various criteria these injuries can be defined as either primary or secondary injury. A primary injury is an initial rapid response to low temperature which causes a dysfunction in the plant cell membranes, metabolic processes, or cell organisation (e.g. cytoskeleton). This class of injury can be reversed if temperature is raised to a non-chilling temperature. Long term exposure to low temperature leads to the development of secondary injury or injuries which are consequent upon primary injuries. For example in response to chilling cell

membranes will undergo phase transitions that will result in various dysfunctions, such as a loss of semi-permeability, incorrect functioning of bound proteins, loss of specificity of membrane-bound signalling systems etc. In the short term the consequences of these are reversible, but in the longer term the oxidation of cell components (eg membranes, proteins) and metabolic disorder is sufficiently severe that it leads to cell death by necrosis.

Generally, the appearance of chilling injury symptoms is associated with the occurrence of oxidative stress. The oxidative reactions result in a dysfunction in cell membranes by generation of AOS, formed by metabolic processes. The most common AOS forms is $O_2^{\bullet-}$ ions and H_2O_2, which are formed in vivo by a variety of mechanisms. Some metabolic enzymatic reactions in plant cells (e.g. xanthine oxidase and urease) will form $O_2^{\bullet-}$.H_2O_2 may be formed by dismutation of two $O_2^{\bullet-}$ ion, by β-oxidation of fatty acids in the course of lipid metabolism and in response to stress. Another source of generation AOS is by pathogen infection. Other sites in plant cells can produce AOS forms, as has been reported in several studies i.e. in the inner mitochondrial membrane and cell wall, by enzymatic reactions in the cell membrane. The increasing steady-state levels of AOS during long-term storage may also be due to the decreasing effectiveness of defence systems based on enzymatic and nonenzymatic antioxidants. Nonenzymatic antioxidants include ascorbic acid (ASC), α-tocopherol (α-TOC) and β -carotene (β -CAR). These molecules are often linked with antioxidant enzymes such as ascorbate peroxidase, glutathione reductase, which regenerate the antioxidant capacity of the quenching molecule. Purely enzymatic reactions, such as superoxide dismutase (SOD), Catalase (CAT), and peroxidase (APX) protect the plant cell by directly scavenging $O_2^{\bullet-}$ and H_2O_2. The physiological changes are summarised in Chapter 2.

All experiments were conducted on two Egyptian varieties (Zibdia and Hindi Be-Sennara) which were used as mango fruit models. Three different maturity stages at time of harvest, immature, half-mature and fully mature fruits were used in this work. Storage conditions were set to 5 different temperatures (2, 4, 7, 10 and 20^{o}C) for 35 days. In all the experiments fruit samples were taken at time intervals of five days. During a range of experiments the effect of chilling was studied on the water and lipid-soluble antioxidants compounds and anti-oxidant enzymes,, various chilling injury symptoms (electrolyte leakage, skin chlorophyll content, chlorophyll fluorescence responses, and a visual injury assessment), and mechanical properties of the fruits. Where appropriate, both the peel and pulp portions of the fruits were investigated. The purpose of these studies was to examine how well different assays of chilling injury worked, how chilling changed

the biochemical properties of the fruits, how these changes correlated with measurable indices of chilling injury, and finally which to ascertain by means of correlation studies which antioxidant components appeared most important in relation to the occurrence of chilling injury.

Chapter 3 describes the possibilities of using chlorophyll fluorescence parameters (F_v/F_m, F_0 and F_m) to assess chilling injury of fruits with the aim of predicting chilling injury symptoms before they are visible. An attempt has been made to analyze the effect of storage temperature, conducted on different mango varieties at different maturity stages. Fruits were stored at 5 temperatures (2, 4, 7, 10 and 20^o) for 35 days. Chilling injury index (CI-index) and chlorophyll *a, b* content and *a/b* ratio were measured every 5 days and chlorophyll fluorescence (CF) every 3 days. It is shown that temperature and variety had a significant effect with storage time, but no effect of maturity stage was observed. A higher storage temperature decreased F_v/F_m and F_m, whereas F_0 increased during the experiment. In prolonged storage at low temperature, Fv/F_m decreased more rapidly up to the end of the experiment in Zibdia than Hindi fruits. In Zibdia, it decreased after 3 days, compared to 6 days in Hindi, and before visible chilling injury symptoms appeared. Also, Zibdia presented higher severity of chilling injury symptoms during storage than Hindi fruits in all maturity stages. CI appeared on day 10 in both varieties at all maturity stages. It was less severe in M1 fruits than M2 and M3. Chl_a degraded more rapidly in Zibdia than Hindi and more rapidly than Chl_b in all maturity stages. It was higher in Zibdia than Hindi. This relationship allows a prediction of CI before visible symptoms appear. The results show that Zibdia fruits are more sensitive to low storage temperatures than Hindi fruits. Therefore, it is necessary to study which factor increases resistance to chilling temperatures.

The effect of storage temperatures on water and lipid-soluble antioxidants such as ascorbic acid (ASC), α-tocopherol (α-TOC) and β-carotene (β-CAR) content during storage and its correlation with CI-index is described in Chapter 4. The experiment was conducted on two mango varieties ‘Zibdia’ and ‘Hindi’, which were harvested in three different maturity stages. The fruits were stored at different storage temperatures, focusing on their response to low temperature, with the aim of achieving a better understanding of the underlying physiological processes of CI development with regard to antioxidants which provide a protection against harmful active oxidative species (AOS). Physiologically, long-term storage increases the imbalance between the oxidant/antioxidant, which leads to increased oxidative damage to cell membranes following reactions with membrane lipids and proteins, which finally results in cell death. Therefore, water and lipid-soluble antioxidants were measured in fruit components (peel and pulp) during the storage

period. Generally, a higher initial ASC content of both fruits was observed in M1 fruits compared with M2 and M3 fruits. α-TOC and β-CAR showed the opposite trend. The lowest level of ASC was observed in almost completely damaged fruits. Rapid decreases in ASC, was observed in half and fully mature fruit and when the fruits were stored at the low temperature range. A negative linear relationship was found between water/lipid-soluble antioxidants and CI-index which proves that water/lipid-soluble antioxidant decreased based on storage temperature, time and fruit maturity stages.

The data obtained in the previous chapter allows us to study the activities of antioxidant enzymes (SOD, CAT, APX and GR) and the terminal products of AOS oxidation of lipids and proteins that occurs during storage. In this experiment, Zibdia and Hindi fruits were stored at two storage temperatures (4 and 10^{o}C) for 35 days. The results indicate that lipid peroxidation and protein oxidation increase up to end of the experiment in both varieties and fruit parts. The activity of the antioxidant enzymes increased to the maximum, dependent on storage temperature, variety, maturity stage and time. In addition, fruit parts presented different enzyme activities as to (SOD, CAT, APX and GR) because of storage temperature effects. A negative linear relationship between MDA content and the activities of antioxidant enzymes (CAT and APX) was observed. This relationship shows the imbalance between antioxidant activity and AOS formation, which is reflected by increasing lipid peroxidation in both mango varieties and fruit parts. Similarly, a negative linear relationship between protein oxidation (PCG) and antioxidant enzyme activities (CAT, APX and SOD) was found.

Chapter 5 presents the development of physiological changes such as ethylene (C_2H_4) production, respiration rate (CO_2), firmness and ion leakage on mango cultivars. The results indicated that the climacteric respiration peak was observed on day 10 of storage. In addition, ethylene production reached a maximum on day 5 of storage. M3 fruits produced the highest amount of C_2H_4 compared to M1 and M2 fruits in both varieties. The same pattern was observed with respiration rate. Storage temperature affects C_2H_4 production and respiration rate (CO_2), which are higher at 20^{o}C than chilling temperatures (2 to 10^{o}C). Storage at low temperature kept fruit firmness higher in both varieties. Hindi fruits presented a higher firmness than Zibdia fruits and M1 fruits were firmer than M2 and M3. Another effect of storage temperature observed in this experiment is that ion leakage increased with lower storage temperatures, which indicates the dysfunction in the membranes and walls of the plant cell. A relationship between lipid peroxidation products (MDA) and ethylene production, respiration rate, firmness and ion leakage was observed. These relationships indicate the dysfunction of cell membrane due to increased peroxidation processes in the

membrane. MDA had a negative relationship with ethylene (C_2H_4) production and fruit firmness. It is clear from the relationships that the storage temperature plays an important role in increasing lipid peroxidation processes and inhibiting the hydrolytic enzymes, which are related to fruit softening. On the other hand, a negative relationship was found between MDA and respiration rate (CO_2) and ion leakage. The results indicate that storage temperature causes a loss of function in cell membranes by enhancing lipid peroxidation. A further possibility is to sort the biological differences of mango fruits to increase the shelf life.

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Samenvatting

Momenteel stijgt de waarde en de productiehoeveelheid van mangovruchten wereldwijd. Vele studies benadrukken hoe de koudeschade de kwaliteit van tropische vruchten, zoals mango, beïnvloeden tijdens naoogst behandeling, vervoer en bewaar. Aangezien de mango één van de meest gewaardeerde en populaire vruchten in de wereldmarkt en een climacterische vrucht is, d.w.z. snel rijpend na de oogst, maakt het essentieel te onderzoeken hoe de bewaar de externe en interne fruitkwaliteit beïnvloedt. Over het algemeen worden lage temperaturen gebruikt om metabolische processen te vertragen tijdens bewaar om zodoende het uitstalleven te verlengen. De mango vrucht wordt gekenmerkt door een korte houdbaarheid bij ongekoelde omstandigheden, maar het is ook gevoelig voor lage bewaartemperaturen. De gevoeligheid van mangovruchten voor lage bewaartemperaturen (i.e. onder 12^{o}C) gepaard aan hun bederfelijkheid bij ongekoelde bewaring beperkt de behandeling, de bewaring en het vervoer van de vruchten. Om deze redenen moeten de voor- en naoogst factoren die van invloed zijn op de gevoeligheid van mangovruchten voor kouschade zorgvuldig worden bestudeerd om het uitstalleven en de kwaliteit van de vruchten te verbeteren.
Het belangrijkste doel van deze studie was de gevolgen te kwantificeren en te begrijpen van bewaarcondities op het voorkomen van kouschade in mangovruchten tijdens gekoelde bewaring op lange termijn. Veel aandacht is besteed aan de integratie van factoren die tot de gevoeligheid van kouschade zouden kunnen bijdragen en er is geprobeerd om een beter inzicht in de fysiologische processen te krijgen die met kouschade samenhangen, opdat kouschade beter begrepen en hopelijk voorspeld kan worden. Het onderzoek richtte zich op het effect van bewaaromstandigheden (temperatuur en duur van de bewaring), op mango cultivars (Zibdia en Hindi Be-Sennara), geoogst in de verschillende stadia van de fruitrijpheid, onrijpe (M1), half-rijp (M2) en volledig rijp (M3), met betrekking tot de verschijning van kouschade.

Hoofdstuk 1 geeft een overzicht van mango als plant, gewas, en product in zowel internationale als lokale markten.

Fysiologisch gezien is het effect van lage temperaturen op plantcellen een fysieke en/of fysiologische verstoring, of verwonding, in celmembranen en het cytoplasma. Op basis van diverse criteria kunnen deze verwondingen als primaire dan wel als secundaire verwonding worden gezien. Een primaire verwonding is een eerste snelle reactie op lage temperatuur die een disfunctie veroorzaakt in de membranen van de plantencel, in de metabolische processen, of de celorganisatie

(b.v. cytoskelet). Dit type verwonding kan hersteld worden als de temperatuur weer boven de schade drempel wordt gebracht. De lange termijn blootstelling aan lage temperatuur leidt tot de ontwikkeling van secundaire schade of verwondingen die uit een samenstelling van primaire verwondingen voortvloeien. Bijvoorbeeld als reactie op het koelen van de cel zullen de membranen faseovergangen vertonen die in diverse disfuncties zullen resulteren, zoals een verlies van semi-doorlaatbaarheid, het onjuist functioneren van gebonden eiwitten, het verlies van membraan gebonden specifieke signaal transductie systemen enz. Op de korte termijn zijn de gevolgen hiervan omkeerbaar, maar op langere termijn zal de oxidatie van celcomponenten (b.v. membranen, enzymen) en de metabolische wanorde zo groot zijn dat het tot celdood door necrose leidt.

Over het algemeen, zal de verschijning van kouschade gepaard gaan met oxidatieve schade (stress). De oxiderende reacties resulteren in een disfunctie in de celmembranen door het genereren van AOS (Active Oxygen Species = actieve zuurstof agentia), oorspronkelijk gevormd door metabole processen. De meest voorkomende vormen van AOS zijn $O_2^{\bullet-}$ ionen en H_2O_2, die *in vivo* gevormd worden door een verscheidenheid aan reakties. Sommige metabole enzymatische reacties in plantcellen (bijv. de xanthine oxidase en urease) zullen $O_2^{\bullet-}$ vormen. H_2O_2 kan door dismutatie van twee $O_2^{\bullet-}$ ionen worden gevormd, door β-oxidatie van vetzuren tijdens lipide metabolisme en als gevolg van stress. Een andere bron van AOS productie is door ziekteverwekkende organismen. Andere delen van de plantencel kunnen AOS produceren, zoals in verscheidene studies is beschreven, zoals in het mitochondriele binnenmembraan en in de celwand, maar ook door enzymatische reacties in de celmembraan. De voortdurende stijging van AOS-niveaus tijdens bewaring op de lange termijn kunnen ook toegeschreven worden aan de dalende doeltreffendheid van defensiesystemen, die op enzymatische en non-enzymatische anti-oxidanten zijn gebaseerd. Non-enzymatische anti-oxidanten zijn o.a. ascorbinezuur (ASC), α-tocoferol (α-TOC) en β - caroteen (β - CAR). Deze moleculen zijn vaak verbonden met antioxidant enzymen zoals ascorbaat-peroxidase, glutathion-reductase, welke antioxidant capaciteit van de reducerende moleculen regenereren. Zuiver enzymatische reacties, zoals superoxide dismutase (SOD), katalase (KAT), en de peroxidase (APX) beschermt de plantcel door $O_2^{\bullet-}$ en H_2O_2 direct af te vangen. De fysiologische veranderingen worden samengevat in Hoofdstuk 2.

Alle experimenten werden uitgevoerd op twee Egyptische rassen (Zibdia en Hindi Be-Sennara) die als modellen voor mangofruit werden gebruikt. Drie verschillende rijpheidstadia op het oogsttijdstip, onrijp, halfrijp en volledig rijpe vruchten werden gebruikt in dit onderzoek. Er werd 35 dagen lang bewaard bij 5 verschillende temperaturen (2, 4, 7, 10 en 20^{o}C). In alle experimenten werden de

metingen gedaan met tussenpozen van vijf dagen. In een aantal experimenten werd het effect van het koelen bestudeerd op de water- en lipide-oplosbare anti-oxidanten en hun enzymen. Diverse kouschade symptomen (zoals elektrolytlekkage, de hoeveelheid chlorofyl in de schil, de chlorofylfluorescentie reacties, en een visuele schade beoordeling), en mechanische eigenschappen van de vruchten werden bestudeerd. Wanneer dat mogelijk was werden zowel de schil als het vruchtvlees onderzocht. Het doel van deze studies was te onderzoeken hoe goed de verschillende beoordelingen van kouschade werkten, hoe het koelen de biochemische eigenschappen van de vruchten veranderde, hoe deze veranderingen met meetbare indexen van kouschade correleerden, ten einde door middel van correlatiestudies na te gaan welke anti-oxidanten het belangrijkst waren voor het ontstaan van kouschade.

Hoofdstuk 3 beschrijft de mogelijkheden om de Chlorofylfluorescentie parameters te gebruiken (F_v/F_m, F_0 en F_m) om de kouschade van vruchten vast te stellen met het doel de kouschade symptomen te voorspellen alvorens zij zichtbaar worden. Er is een poging gedaan om het effect van bewaartemperatuur op verschillende mango rassen in verschillende rijpheidstadia te analyseren. De vruchten werden 35 dagen bewaard bij 5 temperaturen (2, 4, 7, 10 en 20^{o}C). De kouschade index (CI-index) en de concentratie chlorofyl *a, b* en de chlorofyl *a/b* verhouding werden elke 5 dagen gemeten en de chlorofylfluorescentie (CF) om de 3 dagen. Er werd aangetoond dat de temperatuur en de rassen met de bewaartijd een significant effect hebben, maar er werd geen effect van rijpheidstadium waargenomen. Een hogere bewaartemperatuur verminderde F_v/F_m en F_m, terwijl F_0 tijdens het experiment steeg. Tijdens een verlengde bewaring bij lage temperatuur verminderde de F_v/F_m waarde sneller, aan het eind van het experiment, in Zibdia dan in Hindi vruchten. In Zibdia nam het na 3 dagen af en in Hindi na 6 dagen en dit alles vóór de zichtbare symptomen van kouschade konden worden waargenomen. In alle rijpheidstadia liet Zibdia zwaardere kouschade symptomen tijdens bewaring zien dan Hindi. Kouschade verscheen op dag 10 in beide rassen in alle rijpheidstadia. Het was minder zwaar in M1 vruchten dan in M2 en M3. Chl_a brak sneller af in Zibdia dan in Hindi en sneller dan Chl_b in alle rijpheidstadia. Er verdween meer chlorofyl in Zibdia dan Hindi. Deze verhouding staat een voorspelling van kouschade toe voordat de zichtbare symptomen verschijnen. De resultaten tonen aan dat de Zibdia vruchten gevoeliger zijn voor lage bewaartemperaturen dan Hindi vruchten. Daarom is het noodzakelijk te onderzoeken welke factoren de weerstand tegen lage temperaturen verhoogt.

Het effect van bewaartemperaturen op water- en lipide-oplosbare anti-oxidanten, zoals ascorbinezuur (ASC), α-tocoferol (α-TOC) en β-caroteen (β-CAR)

concentraties tijdens bewaring en de correlatie met CI-Index wordt beschreven in Hoofdstuk 4. Het experiment werd uitgevoerd op twee mango rassen `Zibdia' en `Hindi', welke in drie verschillende rijpheidstadia werden geoogst. De vruchten werden opgeslagen bij verschillende lage bewaartemperaturen, met het doel een beter inzicht in de onderliggende fysiologische processen van de ontwikkeling van kouschade met betrekking tot anti-oxidanten die een bescherming tegen schadelijke actieve oxiderende reagentia (AOS) te krijgen. Vanuit fysiologisch perspectief verhoogt de langdurige bewaring de uit evenwicht situatie tussen oxiderende en de reducerende reagentia, welke tot verhoogde oxidatieve schade aan celmembranen, na reacties met membraan lipiden en eiwitten, leidt. Dit resulteert definitief in celdood. Daarom werden gedurende de bewaarperiode water- en het lipide-oplosbare anti-oxidanten gemeten in vruchtdelen (schil en vruchtvlees). Over het algemeen werd van beide rassen een hogere begin ASC concentratie in M1 vruchten waargenomen in vergelijking met de M2 en M3 vruchten. α-TOC en β-CAR toonden de tegenovergestelde tendens. Het laagste niveau van ASC werd waargenomen in bijna compleet beschadigde vruchten. Een snelle dalingen van ASC, werd waargenomen in de half (M2) en volledig rijp (M3) fruit en bij de vruchten die bij de lage temperaturen werden opgeslagen. Een negatieve lineaire verhouding werd gevonden tussen water/lipide-oplosbare anti-oxidanten en de CI-Index hetgeen bewijst dat water/ lipide-oplosbare anti-oxidanten af nemen op basis van de bewaartemperatuur, de bewaarduur en het rijpheidstadium.

De gegevens die in het vorige hoofdstuk werden verkregen staan ons toe om de activiteiten van anti-oxidant enzymen te bestuderen (SOD, CAT, APX en GR) en de eindproducten van AOS oxydatie van lipiden en eiwitten die tijdens bewaring voorkomen. In dit experiment werden Zibdia en Hindi 35 dagen opgeslagen bij twee bewaartemperaturen (4 en 10^{o}C). De resultaten wijzen erop dat de lipide-peroxidatie en de eiwit-oxidatie in beide rassen en in alle vruchtdelen tot aan het eind van het experiment toenemen. De activiteit van de anti-oxidant enzymen steeg tot het maximum, afhankelijk van de bewaartemperatuur, de rassen, het rijpheids stadium en de bewaarduur. Bovendien werden in de verschillende vruchtdelen verschillende enzymactiviteiten voor SOD, CAT, APX en GR, in relatie tot de bewaartemperatuur. Er werd een negatief lineair verband tussen de MDA concentratie en de activiteit van anti-oxidant enzymen (KAT en APX) waargenomen. Dit verband toont de onevenwichtigheid tussen anti-oxidant activiteit en de AOS vorming, welke door stijgende lipideperoxidatie in zowel mango rassen als vruchtdelen wordt weerspiegeld. Analoog werd een negatief lineair verband tussen eiwitoxidatie (PCG) en anti-oxidant enzymactiviteiten (KAT, APX en SOD) gevonden.

Hoofdstuk 5 beschrijft de ontwikkeling van fysiologische veranderingen zoals ethyleenproductie (C_2H_4), ademhalingssnelheid (CO_2), stevigheid en ionenlekkage in mango cultivars. De resultaten wezen erop dat de climacterische piek in de ademhaling op dag 10 van de bewaring werd waargenomen. Bovendien bereikte de ethyleen productie een maximum op dag 5 van bewaring. M3 vruchten genereerden de hoogste hoeveelheden C_2H_4 in vergelijking met M1 en M2 vruchten in beide rassen. Hetzelfde patroon werd waargenomen t.a.v. de ademhalingssnelheid. De bewaartemperatuur beïnvloedt de C_2H_4 productie en de ademhalingssnelheid (CO_2), welke hoger zijn bij 20^{o}C dan bij lage temperaturen (2 aan 10^{o}C). Bewaring bij lagere temperaturen behield de vrucht stevigheid beter bij beide rassen. Hindi vruchten waren steviger dan Zibdia vruchten en M1 vruchten waren steviger dan M2 en M3. Een ander effect van de bewaartemperatuur dat in dit experiment werd waargenomen is dat de ionenlekkage bij lagere bewaartemperaturen toenam, dit duidt op het disfunctioneren van de membranen en de cel wanden in de plantcel. Er werd een verband waargenomen tussen de producten van de lipide peroxidatie (MDA) en de ethyleenproductie, ademhalingssnelheid, de stevigheid en de ionenlekkage. Deze verhoudingen wijzen op het disfunctioneren van het celmembraan, wat toe te schrijven is aan verhoogde peroxidatie processen in het membraan. MDA had een negatieve relatie met ethyleen (C_2H_4) productie en de vruchtstevigheid. Uit deze verhoudingen is af te leiden dat de bewaartemperatuur een belangrijke rol vervult in de toename in lipide peroxidatie processen en het remmen van de hydrolytische enzymen, welke in verband staan met het zacht worden van de vrucht. Hoewel anderzijds een negatieve relatie werd gevonden tussen MDA en de ademhalingssnelheid (CO_2) en de ionenlekkage. De resultaten wijzen erop dat de bewaartemperatuur een verlies van functionaliteit in celmembranen veroorzaakt door lipide peroxidatie processen te intensiveren. Een andere mogelijkheid is het sorteren van de biologische verschillen van mangovruchten om het uitstalleven te verhogen.

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المـلخص العـــربى

المقدمة:

تعتبرالمانجو من المحاصيل المناطق الاستوائية وذات قيمة اقتصادية في السوق العالميه و المحليه أيضا وقد أشارت الدراسات ان المانجوتعتبر حساسة لأنخفاض حرارة التخزين على فترات طويله وأن تخزين ثمار المانجو على دراجات حرارة اقل من ١٢م الى ظهور ظاهرة اعراض (Chilling injury) البرودة على الثمار مما يؤدى الى تحديد عمليات التداول و النقل او الشحن وكذلك عملية التسويق. كما تحدث هذة الظاهرة فى مراحل مختلفة من مراحل المعاملات بعد الحصاد مما يؤثر على الجوده المنتج. فسيولوجيا يؤدى التخزين على دراجات منخفضة الى توليد جزيئات اكسجين نشطة (radicals Free). التى تتحد مع مختلف المركبات العضوية داخل الخلية النباتية. وكما تتولد هذة الجزيئات فى كلا من الخلية النباتية و الحيوانية. تتولد هذة الجزيئات من مناطق مختلفة داخل الخلية النباتية عن طريق التفاعلات الأنزيمية و غيرالأنزيمية نتيجة المؤثرات الخارجية على الخلية. المؤثرات التى تساعد على توليد هذة الجزيئات مثل الملوحة, الجفاف, أنخفاض او ارتفاع درجة الحرارة. الاماكن التى تتولد فيها هى الجدر الخلوية, الاغشية البلازمية, الميتوكوندريا, الكلوروبلاست, السيتوبلازم. وبخصوص موضوع البحث المطروح هو أن تخزين المأنجوعلى درجات حرارة منخفضة ولمدد طويلة يساعد على زيادة توليد Free radicals ومع استمرار التخزين يؤدى الى زيادة مستوى هذة Free radicals داخل الخلية اذ تتفاعل مع البروتين و الدهون الاغشية البلازمية مما تفقد خصصائصها ووظائفها فى مراحل متقدمة من التخزين. ويوجد اشكال مختلفة من الجزيئات الاوكسسجينية مثل: ($NO^{\bullet}$,H_2O_2, $O_2^{\bullet-}$, and $HO^{\bullet}$) تتفاعل الجزيئات مع اهم مركبات الاغسية البلازمية البروتين و الدهون والمكونة لها وايضا تتحد مع (DNA) فى سلسلة من التفاعلات. نواتج هذة التفاعلات يمكن تقديرها وقياسها كمؤشرات دالة على جدوث ما يعرف بالتفاغلات الاكسدة (Oxidative Stress). الدهون تعتبر الهدف الاول فى تفاعلات الاكسدة التى تنشط خلال تخزين الثمار. نتيجة لاستمرار التخزين على درجة حرارة منخفضة تستمرهذة التفاعلات مما يؤدى الى موت الخلية, وبالتالى تظهر الاعراض. لوقف تلك التفاعلات الاكسدةحيث يلزم الخلية الى وجود مضدات الااكسدة (Antioxidants) ومنها المركبات أنزيمية مثال (antioxidant enzymes: SOD, CAT, APX and GR) وغير أنزيمي (non-enzymatic: Ascorbic acid, α-Tocopherol and β-Carotene) اذ تتفاعل مع Free radicals لتقليل تزايد مستواها داخل الخلية. وجود التوازن ما بين مستوى Free radicals و تركيز antioxidants تبقى الخلية حية وأختلال هذا التوازن نتيجة لأى مؤثر مثل حرارة التخزين تبدأ تفاعلات الأكسدة Oxidative Stress وظهور أعراض البرودة.

تهدف الدراسة هنا الى تحديد التحليل الكمى وتفهم تأثير ظروف التخزين على حدوث ظاهرة (Chilling injury) خلال التخزين لفترات طويله على دراجات حراره منخفضه أقل من 12^{o}م. كما تهدف الدراسة الى الأهتمام بالعوامل التى قد يكون لها علاقه بظهور وتطور هذه الظاهره. ولتفهم هذه الظاهرة يتطلب الى التفعلات الحيوية التى تحدث خلال التخزين والتى تؤدى الى ظهور أعراض الاصابة وذلك للتنبأ بحدوث هذه الظاهرة. وكان التركيز على العوامل التى لها دور كبير هى درجة حرارة وقت التخزين على ثمار المانجو.

ولدراسة هذة الظاهرة يتطلب الى أجراء بحث على احد محاصيل الفاكهة المناطق الأستوائية مثل المأنجو وهى موضوع الدراسة والمعروفة بأهميتها الأقتصادية على المستوى السوق الدولى و العربى و

المصرى. والأتجاه هو أجراء بعض التجارب للتقيم بعض الأصناف المأنجو المصرية مثال الذبدية و الهندى بسنارة لمعرفة مدى المقاومه او الحساسية للتخزين على درجات منخفضة و كذلك التنبؤ بحدوث Chilling injury قبل ظهور الأعراض الأصابة. وايضا لمعرفه مستوى (Ascorbic acid, α-Tocopherol and β-Carotene) وكذلك نشاط (antioxidant enzymes: SOD, CAT, APX and GR). وكذلك قياس نواتج تفاعلات الأكسدة Oxidative Stress. وتمت التجارب على المأنجو التى تم حصادها على ثلاث أعمار مختلفه (Immature, Half mature and Full mature fruits) وتم تخزينها على خمس درجات حرارة (2, 4, 7, 10, 20°م) لفترة 35 يوم لتتبع كل القياسات المختلفة المطلوبة كل حمس أيام من الفترة الكلية للتخزين وفى أجزاء مختلفة من الثمرة (القشرة و اللب).

اولأ: أستخدام تقنية قياس نشاط الكلورفيل Chlorophyll Fluorescence (CF)

استخدام هذة التقنية بهدف تقيم مدى الحساسية للتخزين وكذلك التنبؤ بحدوث ظاهرة Chilling injury قبل ظهور الأعراض الأصابة كما تم وصفة فى الجزء الثالث. أذ أنه تم تحديد مدى الحساسية أصناف المأنجو لأنخفاض درجة حرارة التخزين. تمت هذة الدراسة صنفين من المأنجو (المزروعة تحت الظروف المصرية). تم قطف الثمار الصنفين (الذبدية و الهندى بسنارة) على ثلاث أعمارمختلفة. ورعى بعض الاجراءات اثناء القطف (اختيار الاشجار و الثمار) كذلك الاحتياطات المطلوبة فى نقل الثمار وشحنها الى المملكة الهولندية. ومن واقع النتائج المتحصل عليها أن لوحظ أن نشاط CF أنخفض بشدة بعد 3 أيام فى الذبدية و 9 أيام الهندى و خصوصا عند تخزينهما على 2 ْ م و4 ْم بخلاف التخزين على 7 ْ م و10 ْم اما التخزين على 20 ْم كأن الأنخفاض شديدا.كما لوحظ أن صنف المأنجو " الذبدية" حساس للتخزين على درجة حرارة منخفضة بخلاف " الهندى" وذلك واضح من القياسات المأخوذة خلال فترة التخزين. وأيضا لوحظ أن اعراض الاصابة Chilling injury تبدأ عند اليوم العاشر من التخزين. تطور هذة الاعراض بزيادة فترة التخزين. وبربط النتائج المتحصل عليها أن كل من CF و Chilling injury index يتضح أن صنف الزبدية شديد الحساسية للتخزين على درجات الحرارة المنخفضة عن الهندى. كما تختلف شدة الأصابة بأعراض البرودةعلى حسب الصنف وكذلك العمر الثمار. حيث كأنت الاصابة شديدة فى العمر الثالث (Full mature) عن العمر الثأنى (Half mature) و الاول (Immature) فى كلا الصنفين ولكن الاصابة شديدة فى الذبدية عن الهندى. ومن واقع النتائج هذة يمكن القول بأن صنف الذبدية حساس اكثر من صنف الهندى. واذ يمكن التنبؤ بحدوث الأصابة من خلال الأنخفاض الشديد فى CF قبل ظهور الاعراض المرئية على الثمار. وعلى ضوء هذة النتائج بأن الاخلافات بين الاصناف و الأعمار يتطلب الى أجراء تجارب اعمق للدراسة الفرق الفسيولوجى من خلال دراسة مستوى Antioxidants.

ثأنياً: مضادات الأكسدة الغير أنزيمية Non-enzymatic antioxidants

دراسة مستوى مضادات الأكسدة فى كل من الذبدية و الهندى يهدف الى فهم علاقة مستواها وظهور و تطور أعراض البرودة حلال فترة التخزين (35 يوم). من هذة المضادات الاكسدة Ascorbic acid المعروف بـفيتامين C و α-Tocopherol (فيتامين E) و β-Carotene (فيتامين A) لما لهم من دور كبير فى خفض مستوى التفاعلات الاكسدة اثناء التخزين. تمت هذة الدراسة على كل من الصنفين المأنجو موضوع الدراسة وعلى أعمار مختلفة. تم تخزين الثمار على خمس دراجات حرارة (2, 4, 7, 10, 20°م) لفترة 35 يوم. تم قياس مستوى Antioxidants كل خمس أيام منفترة التخزين كما تم

قياسها فى اجزاء مختلفة من الثمرة (القشرة و اللب) وذلك لعمل خريطة لتوزيع محتوى هذة المضادات وعلاقتها مدى تطور أعراض البرودة بين الاصناف والأعمار أيضا.
تدل النتائج المتحصل عليها من هذة التجربة أنه يوجد أختلاف بين الذبدية و الهندى فى محتوى Antioxidants الثلاثة موضوع البحث كذلك لوحظ اختلاف بين أعمار الصنف الواحد.أذ لوحظ ان محتوى الاعمار الاولى (immature fruits) محتواها من Ascorbic acid عالى من العمر الاول و الثانى (Half and full mature fruits) بخلاف محتوى α-Tocopherol و β-Carotene أذ يكون أقل فى العمر الاول ويزداد فى العمر الثانى واعلى فى الثالث. كما لوحظ أن الذبدية فى العموم على محتوى أعلى من Ascorbic acid عن الهندى وفى جميع الاعماروكذلك مستوى β-Carotene اما بالنسبة الى
α-Tocopherol فمحتوى أعلى فى ثمار الهندى عن الذبدية. ومن الملاحظ أن مستوى هذة المضادات يقل بزيادة فترة التخزين الثمار. وايضا لوحظ من واقع النتائج أن ظهور اعراض الاصابة اقل فى العمر الاول عن الأعمار الاخرى وهذا مع أن انها تحتوى على تركيز اعلى من Ascorbic acid وتركيز اقل من
α-Tocopherol وβ-Carotene, بعكس العمر الثانى و الثالث فى كلا الصنفين. الملاحظ هنا و من خلال هذا الجزء انه توجد اختلافات بيولوجية بين الاصناف وكذلك بين الاعمار: لماذا ثمار العمر الاول اظهرت اصابة اقل (immature fruits) عن الاعمار الاقتصادية (الثانى و الثالث)؟ وهل هذا راجع الى اختلاف المحتوى من(Ascorbic acid α-Tocopherol و β-Carotene) بين الاعمار. هذة الاختلاف قد تكون لها مؤشرات عن امكانية استخدامها فى تقليل شدة الاصابة بـ Chilling injury او ذيادة فترة التخزين الى اطول مدة ممكنة للثمار بدون اى اصابة.

ثألثاً: مضادات الأكسدة أنزيمية Antioxidants enzymes

من النتائج المتحصل عليها من التجارب السابقة الزمت الى الاتجاه الى دراسة نشاط مضادات الاكسدة الانزيمية مثل (SOD, CAT, APX and GR) بهدف تفهم نشاط هذة الانزيمات قبل و بعد ظهور الأصابة و امكانية ربط ذلك النشاط بمحتوى خلال فترة التخزين الكلية للثمار. اتمام هذة التجربة من منطلق التجارب السابقة بأن التخزين على درجة حرارة 4°م يسبب اصابة شديدة والتخزين على درجة حرارة 10°م هى الامثل فى عمليات النقل و الشحن فكان الاهتمام فى قياس نشاط مضادات الانزيمية فى نفس الصنفين وعلى أعمار مختلفة. خزنت الثمار لمدة 35 يوم مع أخذ عينات من الثمار(القشرة واللب) كل خمس أيام لقياس نشاط الانزيمات. كما أن العلاقات العكسية اظهرت الى وجود عن خلل فى التوازن بين مستوىantioxidant enzymes activity و توليد AOS وهذا واضح من الزيادة الواضحه فى lipid peroxidation خلال التخزين فى كل من الصنفين الذيدية و الهندى وكذلك الاجزاء الثمار (القشرة و اللحم) و مع نفس الاتجاه لوحظت ايضا بين PCG وantioxidant enzymes activity (CAT, APX and SOD).

رابعاً: الاثلين والتنفس والصلابة Ethylene, respiration and firmness and ion leakage

فى الجزء الخامس من الرسالة يتناول التطورات الفسيولوجية مثل انتاج C_2H_4 ومعدل التنفس و الصلابة و النفاذيه. و تشير النتائج أن Climacteric respiration لوحظ ان ذروة التنفس فى اليوم العاشر من التجربة كذلك اعلى معدل انتاج الاثلين فى اليوم الخامس من التجربة. ويختلف معدلات كل من

الاثلين و التنفس على حسب الاعمار الثمار حيث تكون أقل فى العمر الاول و تذداد مع ذيادة العمر الثمرة. كما لوحظ ان التخزين على دراجات الحرارة المنخفضة يحافظ على صلابة الثمار فى كلى الصنفين. أيضا لوحظ الصلابة أعلى فى العمر الاول و تقل مغ ذيادة العمر الثمرة.
ذيادة ion leakage مغ انخفاض درجة الحرارة التخزين مما يشر الى حدوث خلل فى الاغشية البلازمية و الجدر الخلوية أثناء التخزين. و تثبت العلاقات بين lipid peroxidation و الاثلين و التنفس و الصلابة و ion leakage حيث تشر العلاقات الى الخلل فى الاغشية و الجدر يرجع الى ذيادة lipid peroxidation مما يدل على درجة حرارة التخزين تلعب دور فى ذيادة lipid peroxidation وتثبيط انزيمات التحلل (hydrolytic enzymes) والتى لها دور فى ليونة الثمار. و العلاقة العكسيه التى وجدت بين lipid peroxidation و التنفس تشير الى فقد الوظائف الاغشية الخلويه عن طريق تحلل الدهون. أذ يمكن الاستفادة من هذة الاختلافات البيولوجية لثمار المانجو لزيادة العمر التخزين.

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Loay Arafat

Curriculum vitae

Loay Arafat was born in Mansoura city, Egypt on 10th November 1969. After he fished high school in 1989, he started studying Agriculture (Plant production) in, faulty of Agriculture, Mansoura University, Egypt. The B.Sc. qualification was obtained in 1992 with majors in horticulture, agronomy, and pathology and plant protection. After that, he asked by Horticulture Department of faculty to work as a teaching assistance, he took the responsibilities of doing research and teaching within this department. In the same period he started his M.Sc. program in pomology within the Horticulture Department in fruit production under supervision Prof. Iraqi, M; Prof. El-Boray, M. and Dr. Fahmi M. He did an experimental research for M.Sc. degree in Pomology Department, Mansoura University. It is titled 'a comparison between foliar and soil potassium fertilization on Thompson seedless grape. After that, he employed by the same department as an assistance lecturer (Mansoura University in June 1996).

In 1996, he got a scholarship from High Educational Ministry, Cairo to study in field postharvest fruit quality at Wageningen University and Research Centre in Horticulture Production Chains Group, The Netherlands. In 1999, he started his Ph.D. work. He was also contributed to many international symposium and workshops

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