Chapter 2

Review of literature
2. **Review of literature**

Alphonso mango an important cultivar suffers from a physiological ripening disorder called internal breakdown or spongy tissue which renders the fruit unfit for human consumption. Lot of studies has been conducted to look into the problems of internal breakdown in mango. Involvement of calcium and temperature are the major areas of work in the past. However, the earlier workers failed to control the disorder either using calcium or regulating the convective heat. This indicates that the disorder may be a complex process that needs the investigation in many directions.

In this chapter, the available literature relating to the development of spongy tissue in Alphonso mangoes is summarized and the review has been presented under the following headings.

2.1. General characteristics of the spongy tissue.

2.2. Causal factors for the incidence of spongy tissue.

  2.2.1. Physiological factors.
  2.2.2. Horticultural factors.
  2.2.3. Nutritional factors.
  2.2.4. Biochemical factors.

2.3. Possible control measures for the disorder.

2.1. **General characteristics of the spongy tissue.**

This disorder was first noticed in ripe Alphonso mangoes in 1932 by Cheema and Dani (1934), when they opened and examined the best Alphonso mangoes received from valsad district of Gujarat and Ratnagiri district of Maharashtra for export to Europe.
Amin (1967) described 'Spongy tissue' as white sponge like corky tissue, slightly desiccated in nature in the pulp between the skin and the stone of the ripe fruit. Rarely in extreme cases injury can be seen from outside where the skin turns brownish black forming a flat external depression. According to Chhatpar et.al., (1968-69), the main feature of this abnormality is slight desiccation in the center of the tissue surrounded by a soft halo, it occurs between the skin and the stone of the fruit. Subramanyam et.al., (1971), described that the external symptoms of the spongy tissue are not apparent either at the time of picking or at the ripe stage but is visible only when the fruit is cut open. The affected flesh is pale yellow colour, soft, leathery or spongy nature with or without air pockets accompanied by off flavor.

Katrodia (1979) described spongy tissue as a physiological disorder in which the fruit pulp remains unripe because of the unhydrolysed starch due to physiological and biochemical disturbances caused by heat in the mature fruit at pre and post harvest stages. Tare (1977) observed that the damage due to spongy tissue at Dapoli (Ratnagiri) conditions was maximum in the lower part (65.12 %) and minimum in the upper part (4.65 %) and medium in the middle part (30.23 %) of the fruit. The damage near the fruit stone, in the center and near the skin was 55.6 %, 33.3 % and 11.1 % respectively. Also Katrodia (1979 a) reported that in the fruit lower part (opex) was significantly affected (50.5 %) than upper part (11.5 %), middle part (28 %) and the entire fruit (10 %). He noted that the spongy tissue invariably started from the surface of the stone which usually did not touch the inner skin of the fruit.

The damage varies from place to place and season to season depending upon the orchard and agroclimatic conditions. Also irrigation at maturity stage, vigour of tree, stage of maturity at harvest time, canopies of the trees, nutritional imbalance, ecological factors and various enzyme activities during ripening have been implicated with the occurrence of the spongy tissue. (Subramanyam et.al., 1971, Katrodia 1979, Limaye et.al., 1976 and Gunjate et.al., 1982). Since the external symptoms of the spongy tissue cannot be seen until the fruit is cut open. A nondestructive X-ray inspection method has been developed to detect the disorder.
According to Thomas et al. (1993) the X-ray photographs of mangoes having spongy tissue without any external symptoms showed clear indications of mesocarp damage evidenced by dark grey patches corresponding to the internal air cavities in the flesh surrounded by light grey areas representing the healthy flesh. Another non-destructive technique Neutron Magnetic Resonance Imaging is also used to detect and separate the mangoes having spongy tissue prior to packaging for export (Wang and Wang, 1989). Upchurch et al. (1997) reported the use of interactance measurements for detecting internal breakdown in apples. A technique using body transmittance in the 450 to 1050 ηm region was evaluated as non-destructive method for identifying apples with internal breakdown. Apples with internal breakdown absorbed shorter wave lengths of light (<750 ηm) where as light at longer wavelengths (>750 ηm) was absorbed more by unaffected apples.

2.2. Causal factors for the incidence of spongy tissue.

2.2.1. Physiological factors.

(a) Size, weight and specific gravity of the fruit:— Subramanyam et al. (1971) studied the relationship of weight of fruit to the incidence of internal breakdown in Alphonso mango. They observed that fruits weighing around 200gm or less showed a breakdown of 19 % as compared to fruits weighing more than 300gm which showed a breakdown of 47 %. According to Joshi and Limaye (1986), the percentage breakdown due to spongy tissue was increased with an increase in weight of the fruits from 150-200gm (26.67 %) to 350gm (60.0 %). In general percentage breakdown increased with increase in weight of the fruit except in a few cases. According to Shantha krishnamurthy (1980), the extent of internal breakdown increases with the increase in the specific gravity of the fruit. Fruits of specific gravity 1.00 - 1.02 showed 22 % internal breakdown and those of specific gravity more than 1.02 showed 46 % of internal breakdown.
(b) **Effect of age and vigour of tree:** According to Joshi (1975) the incidence of spongy tissue increases with the age of the tree. He observed 31.11 %, 34.44 % and 52.22 % spongy tissue affected fruits on 20, 40 and 70 years old Alphonso trees respectively. However Subramanyam et al., (1971) observed that the age of tree had no relation with the incidence of spongy tissue. Katrodia and Rane (1981) studied the effect of tree vigour on the occurrence and intensity of spongy tissue in Alphonso fruit under Paria (Bulsar) condition during 1976 and 1977. According to their studies the occurrence of the disorder was significantly more in the fruits harvested from vigorous trees (50 %) as compared to those from weak ones (9 %) and as regards to the intensity of the disorder, there were no significant differences.

The fact that the fruits from vigorous trees showed more damage than the weak ones was explained giving two reasons. The vigorous trees having more and dense foliage might have retained more sensible heat for a long period in their umbrella like canopy holding the fruits as if they were in an incubator, while the weak trees could not retain the sensible heat due to their sparse foliage and more free space between the branches. Secondly, the blowing wind also might have helped to some extent in keeping the microclimate milder around the fruits in the weak tree where as in vigorous tree wind usually blew touching the crown or are entrapped in the tree canopy and get heated up.

(c) **Rain, moisture and irrigation in the soil:** Subramanyam et al., (1971) reported that rain one or two weeks prior to the harvest rendered the Alphonso mango fruits more susceptible to the spongy tissue. According to Joshi (1975), relatively more moisture content of the soil rendered the fruits more susceptible to spongy tissue disorder. Katrodia (1979) observed higher percentage of spongy tissue (69.79 %) in the fruits harvested from the irrigated trees. According to him the damage could be attributed to the high atmospheric temperature in combination with high relative humidity under the tree caused more sensible heat which resulted in more damage of fruits.

(d) **Sunlight:** The effect of sunlight did not show any significant role on the development of spongy tissue (Anonymous 1970). Desai (1966) observed that when
atmospheric temperature was high, fruits exposed to direct sunlight were normally affected by this disorder. He further opined that exposure of fruits on western side is more dangerous as the fruit on that side get sunrays for longer period and hence more likely develop the spongy tissue. Gunjate *et al.*, (1982) reported that there was significant difference in the occurrence of internal breakdown due to post harvest exposure of fruits to sunlight for varying periods. As the exposure period increased there was an increase in the occurrence of the disorder. The occurrence was maximum at 120 minutes (100 %) and 240 minutes (100 %) of exposure to sunlight. Contrary to this, Katrodia (1979) reported that direction of sunlight had no effect on the occurrence and intensity of the disorder as most of the trees under shade were also affected by the disorder. He also reported that one hour exposure of fruits to infrared rays at 40.5 ± 0.5°C could produce 100 % occurrence and 20 % intensity of damage.

**Temperature:** Of the several factors reported to influence spongy tissue temperature has received relatively more attention than the others. Desai (1966), reported that the spongy tissue in grafted and country varieties of mango grown in Gujarat when the ambient temperature was higher than 40.5°C. According to Katrodia (1979) the cause for the development of spongy tissue is the heat coming from the soil (when soil surface temperature reaches about 55.8°C due to solar radiation in the months of April and May as convective flux, which first touches the apex (lower part) of the fruit facing towards the soil and it enters the fruit pulp by the way of conduction and touches the stone, where it gets accumulated raising the fruit pulp temperature to the extent of promoting the damage.

The high temperature of the fruit disturbs the normal ripening of the fruit pulp by denaturation of protein which destroys the functions of enzymes (amylase and invertase). As a result of this irreversible phenomenon the affected fruit pulp remains unripe known as spongy tissue pulp or dessicated tissue. Thus transfer of heat by radiation, convection and conduction has greater impact in causing the damage in the fruit pulp. Due to enzymatic thermal denaturation a lot of starch remains unhydrolysed in the affected fruit resulting in unripe spongy tissue pulp (Treshow 1970; Conn and stumpf 1971). Katrodia
(1979) also reported that the artificial production of spongy tissue could be done by putting the mature fruits in the incubator at low temperature (41 ± 1° C) for longer period (72 hrs) and at high temperature (45 ± 1° C) for shorter period (1, 2 and 3 hrs). Similar trend was reported by Sheth (1981). Esquerra, Lizada et.al.,(1990) subjected the “Carabao” mango fruits to vapour heat treatment (VHT) at a pulp temperature of 46°C for 10 minutes and then ripened at an ambient temperature of 26 to 28°C. VHT induced internal breakdown by the presence of spongy tissue in the inner portion of the mesocarp. VHT increased respiration rate reducing the oxygen concentration.

(f) Transpiration:- Shivashankar,K.S and Mathai (1999) investigated the relationship of leaf and fruit transpiration rates with the incidence of spongy tissue in mango cultivars Dashehari (free from spongy tissue) and Alphonso (susceptible to spongy tissue). Leaf transpiration rates were similar in both the cultivars, but the fruit transpiration rate was significantly higher in cv.Dashehari compared to cv.Alphonso. Artificially induced variations in fruits also resulted in significant changes in the incidence of spongy tissue in cv.Alphonso.

The significant and negative relationship observed between the fruit transpiration rate and the spongy tissue suggests that the lower fruit transpiration rate in cv.Alphonso is varietal specific trait which results in the slower movement of water and minerals to the fruits from soil leading to the development of spongy tissue. Lower transpiration rate may also lead to the higher internal temperature especially during the transition between maturity and initiation of ripening. Similar results were also reported in other crops when grown under different humidities (Clarkson 1984, Menzel Kirkby 1987).

g) Ethylene :- The gaseous plant hormone ethylene regulates many aspects of plant growth, development and senescence. It is biologically active in trace amounts and its effects are commercially important in agriculture (Abeles et.al.,1992). According to Jurg.H and Yang. S.F. (1995) the rate of ethylene production by plant tissue is normally low and its production is induced during certain stages of growth and development.
Ethylene controls the ripening of climacteric fruit by coordinating the timely activation of many genes that cause changes in colour, texture, aroma and flavor.

According to Mc.Murchie et al. (1972) the ripening of climacteric fruit is divided into preclimacteric and climacteric stage and the ripening related biochemical changes and the respiratory rates differ in the two stages. Two systems of ethylene production can be distinguished as system 1 and system 2. System 1 is seen in early preclimacteric fruit before the onset of ripening and ethylene production rate is low. System 2 is observed during the climacterium where the rates of ethylene production are high and it is mainly autocatalytic that is the ethylene production is induced by ethylene itself.

According to Adams and Yang (1979) ethylene is synthesized in the methionine salvage pathway via the following route. S-adenosyl-L-methionine → ACC → Ethylene. The pathway has been confirmed in all higher plants where studied. It is established that the rate limiting step in the ethylene production is ACC Synthase (ACS) which catalyses the formation of ACC (1-amino cyclopropane-1-carboxylic acid). The conversion of ACC to ethylene is catalysed by ACC oxidase (ACO). In fruits both ACC synthase and ACC oxidase are induced during ripening and contribute to the regulation of ethylene biosynthesis. A simple, rapid and sensitive method for the quantitative determination of 1-amino cyclopropane-1-carboxylic acid was described by Lizada and Yang (1979). The assay was based on the liberation of ethylene from ACC with NaOCl (sodium hypochlride) in presence of Hg2+; ethylene was assayed by gas chromatography method. The yield was normally 80% and the method was quite specific which can detect as little as 5 pmol of ACC.

The changes in the activity of the enzymes ACC Synthase and ethylene forming enzyme during ripening have been the subject of research in a number of climacteric fruit. Hoffman and Yang (1980) studied the changes of ACC content in ripening fruits avocados, banana and tomatoes in relation to their ethylene production rates. Change in the level of ACC is determined by the rate of ACC synthesis relative to its rate of utilization or degradation. In tomato both ACC level and ethylene production were low in
mature green stage but ethylene production continued to increase as the ripening increases, while the ACC levels remained roughly constant. In very ripe fruit, ACC level increased than in previous stages.

In avocado and banana, the rapid accumulation of ACC during the onset of ripening indicated that the rate of its formation was greater than that of ethylene synthesis. Decrease in the level of ACC indicates that the rate of ethylene synthesis is greater than that of ACC synthesis. Avocado has a much higher rate of ethylene production than banana and thus utilizes more ACC and thus the ACC level drops rapidly during and immediately after ethylene peak. As the fruit becomes overripe the ACC level increases and the ethylene production decreases because the ethylene forming enzyme is impaired as the tissue senesces. In mango a small but notable peak of ethylene is produced during ripening in the mango cultivars Haden, Kent, Manila and Ataulfo (Burg and Burg 1962, Lopez, Gomez et al., 1992, Cua and Lizada 1990).

Cua and Lizada (1990) reported that in Carabao mangoes ethylene was produced prior to full maturity and lower ethylene levels were observed in the inner mesocarp. This may be due to the lower expression of ACC synthase gene. Burg and Burg (1962) also reported that the mature but unripe mangoes had high ethylene levels while they were still attached to the tree and suggested that this ethylene was rendered ineffective by a ripening inhibitor. Marcel and Young (1979) reported that a close relationship exists between the rapid increase in the cell wall depolymerizing enzymes and the rise in respiration and ethylene production in avocado fruits. Also Pesis et al. (1978) found a direct correlation between cellulase activity, softening, respiration and ethylene production in avocado fruits.

Teresa F.W and Elspeth (1992) reported that the rapid softening of kiwi fruit can be induced by treating with ethylene which increase the pectinesterase activity. The rate of softening of the fruit increases by increased deesterification of cell wall pectins, followed by degradation of solubilized pectin. Mitcham and Mc.Donald (1997) studied the effect of post harvest heat treatments on inner and outer tissue of mango fruit. Tommy
atkins mango fruits were subjected to vapour heat treatment and they reported that the ACC oxidase activity was reduced in the inner mesocarp tissue. Keista et.al.,(1998) studied the ethylene synthesis in mango fruit following heat treatment. According to their report inhibition of ethylene production was found during heat treatment and was due to inhibition of both ACC synthase and ACC oxidase. ACC oxidase recovers fully following the heat treatment where as ACC synthase recovers partially but enough to allow the heated fruits to achieve the ethylene peak. From the studies on different enzymes it is evident that the internal breakdown may be due to inhibition of ripening process in the inner mesocarp of the fruit and the lower ethylene synthesis might have lead to the lower activities of many hydrolytic enzymes involved in ripening. Selvaraj et.al.,(2000) also reported the lower activity of ethylene forming enzymes in the spongy tissue of Alphonso mango fruit. The activity was three fold lower in the breakdown tissues when compared to the healthy tissue.

Lad et.al.,(1985) studied the effect of post harvest Ethephon dipping of fruits on the occurrence of spongy tissue disorder in Alphonso mango. It was observed that the post harvest dipping of fully mature Alphonso mango fruits in 500 ppm, 750 ppm and 1000 ppm solution of ethephon minimised the occurrence of spongy tissue disorder. It was probable that the ethylene released by ethephon inside the fruit might interact with the ripening inhibitors which are responsible for the inactivation of the enzymes catalase, peroxidase and amylase during the ripening process (Mattoo et.al.,1968). Very little information is available related to the activity of the ethylene synthesizing enzymes ACC oxidase and ACC synthase in mango fruit.

2.2.2. Horticultural factors.

(a) Varietal factor:- The incidence of the spongy tissue was found more or less in all grafted and country mango varieties. Grown in Gujarat state but the kesar variety was found less susceptible to this disorder (Desai, 1966). Amin (1967), reported that the Jamadar cultivar of Gujarat state was also found susceptible to this malady. Katrodia (1979) studied Rajapuri, Vanraj, Madrasi Afus, Kesar, Hybrid (Alphonso × Baneshan),
Jamadar and country varieties. Among these Rajapuri variety was found resistant to artificial production of spongy tissue even at four hours of sun exposure (11:30 hr to 15:30 hr) at preharvest stage and the rest of the varieties showed different degrees of susceptibilities to this disorder. According to him this variation could be due to different physiochemical properties of the fruit skin which act as an insulating material against heat. According to Limaye et al., (1975) the spongy tissue development is not only restricted to the Alphonso cultivar. They observed its occurrence to the extent of 46.67% in Alphonso while it was 36.67%, 26.67%, 13.33%, 10.0%, 6.67% and 3.33% in Vanraj, Olour, Goamankur, Vellaikolambam, Swarnarekha and Fernandin varieties respectively. The cultivars Pairi, Kesar, Doodhpeda, Neelum and Dashehari were free from spongy tissue.

(b) Root stock:- Joshi (1975) observed that the fruit of Alphonso on Alphonso seedling root stock showed the maximum percentage of damage (57.39%) and those on Vellaikolambam root stock showed the least (35.43%). The remaining root stocks namely Shahabuddin (55.93%), Pairi (48.59%), Neelum (46.16%), mixed seedlings that is control (46.09%), Peshwa (43.05%) and Totapuri (43.06%) also showed slightly varying effect on the occurrence of spongy tissue in fruits. The vigour of root stock had its influence on the occurrence of spongy tissue due to differences in the capacity to absorb the various nutrients from the soil (Joshi and Roy 1985).

(d) Place, time and stage of maturity at harvest:- Subramanyam et al., (1971), found that the percentage of spongy tissue incidence varied from place to place considering the coastal areas and the inland areas. Fruit from the coastal areas Belgaum (Karnataka state), Bulsar (Gujarat state) and Chittur (Andhra pradesh) recorded 57%, 50% and 47% spongy tissue respectively. Whereas the fruits from inland areas Mysore, Bangalore, Dharwar, Ratnagiri and Kolar districts recorded 9%, 26%, 2%, 24% and 5% respectively. These results indicated that the breakdown in the coastal areas is maximum (41%) compared to the inland areas (22%). Joshi (1975) reported that the fruit of tree at the base of the hill showed 56.67% spongy tissue followed by the fruit of tree on the slope (46.6%) and on the top of the hill (35.56%). The occurrence and
intensity vary from place to place because of different agroclimatic conditions prevailing at different locations.

Subramanyam et al., (1971) studied the incidence of spongy tissue in Alphonso mango harvested in three seasons namely early, middle and late season. Fruits harvested in the middle of the season i.e. during the first two weeks of June, recorded higher percentage of breakdown (43 %) compared to early (17 %) and late (37 %) seasons. Joshi and Limaye (1984) observed that the late harvesting renders the fruit more susceptible to spongy tissue. He reported 43.33 % and 53.33 % breakdown in the fruits harvested early and late seasons respectively. Internal breakdown was first observed by Cheema and Dani (1934) while examining the fruit picked at C stage (full) maturity. They further noticed that the tendency for the internal breakdown becomes more at the end of the season. Early picked fruits escaped the incidence of disorder to some extent but the quality of such ripe fruits was inferior. Rangawala (1975), Patkar (1978), Limaye et al., (1976) and Katrodia (1979) observed the similar trends.

Amin et al., (1974), observed that the susceptibility to the spongy tissue development increases with advancing maturity of the fruit. Joshi (1975) studied the occurrence of spongy tissue in relation to the stage of maturity at harvest. He found that in 1974 and 1975, the percentage of spongy tissue at $3/4^{th}$ stage of maturity were 5 % and 10 %, at $7/8^{th}$ stage maturity were 20 % and 23.3 % and at full stage of maturity were 55 % and 53.3 % respectively. Also the tree ripe fruits showed 76.5 % and 85 % of spongy tissue in the year 1974 and 1975 respectively. According to Limaye et al., (1976), about 10 % of fruits were affected by the incidence of spongy tissue when the fruits were harvested at A stage (12 anna), 87.6 % of fruits showed spongy tissue development when harvested at D (tree ripe) stage when the fruits were harvested at B stage (14 anna) of maturity the occurrence of spongy tissue was less about (23.33 %) with an acceptable fruit quality. Fruits harvested at green ripe stage were less affected by spongy tissue when compared to the fruits harvested at the full ripe stage.
(e) Insect pests and microbial factors:- Insect pests do not seem to play any significant role in the development of spongy tissue in Alphonso Mango. Experiments were conducted during 1990 at fruit research station, Navsari, Junagadh and Gandevi on the initiation of infection. Mechanical protection of inflorescences against the insect (mango hoppers) showed no significant role of insects on the incidence of spongy tissue (Anony., 1970). Subramanyam et.al.,(1971) noticed the presence of stone weevil in the stones of both healthy and affected fruits of Alphonso mango to the same degree. Chhatpar et.al.,(1968 – 69) studied the incidence of spongy tissue development in mango with reference to the role of microorganisms. They could isolate Bacillus species from spongy tissue which when innoculated in healthy mango fruits of Alphonso, Neelum, Pairi and Fazli, showed the spongy tissue formation. But one local variety known as Pachhatio did not develop the spongy tissue on inoculation. The isolates were identified as Bacillus macerans, Bacillus ureus, Bacillus subtilis and Bacillus megaterium.

There are contrasting reports about these causal organisms from different workers. Dr.M.K.Patel studied the sections of affected spongy tissue under the microscope and showed that to be full of starch and absence of organisms as reported by Desai (1966). Similar findings were made by Subramanyam et.al.,(1971). According to them, the breakdown of tissue due to bacterial infection (Bacillus species) appears to be doubtful, since pathogenecity has not been clearly established. Low pH and high acidity in the fruit pulp at harvest stage appears to be highly unfavorable for the growth of Bacillus bacteria. However it is likely that the physiological abnormality in the affected tissue predisposes the fruit to secondary bacterial infection at the ripe stage. Katrodia (1979) also ruled out the possibility of the role of bacteria as causal agents in the occurrence of spongy tissue in Alphonso mango.

2.2.3. Nutritional factors.

Most of the work done to understand the causes of internal breakdown were on nutritional aspects of the trees. Amin (1967) Studied the effects of application of Nitrogen, Phosphorous and Potassium fertilizers and microelements like Boron,
separately and in the combined form on the incidence of spongy tissue at fruit research station Mangrol, Junagadh and Gandevi. He observed that soil as well as foliar application of the above elements under irrigated and nonirrigated conditions did not help to overcome the malady in Alphonso.

Similar observations were made by Joshi (1975) when he studied the effect of Nitrogen, Phosphorous and Potassium on spongy tissue development in Alphonso mango at Vengurla. The results of the work showed no consistent effects of different levels of N, P and K on the occurrence of spongy tissue. Also Subramanyam et al., (1971) observed that the fruits harvested from well nourished gardens showed higher percentage of breakdown. Katrodia (1979) in his studies on nutritional status of mango orchard observed that the soil of mango orchard was found to be well supplied with macro elements (N, P, K, Ca and Mg) and micro elements (Zn, Cu, Fe, Mn and B) at fruit set as well as fruit harvest stages indicating that probably the nutrients have no role to play in causing the spongy tissue disorder in Alphonso fruits.

Raymond et al., (1998) studied the relationship between internal breakdown, mineral element concentration and weight of Tommy Atkins mango fruits. According to them in Tommy Atkins mangoes the incidence of internal breakdown had no relation with the fruit weight and with any nutrient element including calcium. Tare (1977) in his studies observed that calcium content in Alphonso leaf increased from 1.63 % at fruit set stage to 1.86 % at fruit harvest stage. Katrodia (1979) studied the concentration of macro and micro elements of leaf and stalk of the spongy tissue affected and unaffected Alphonso fruits and reported that probably the nutritional status of leaf and stalk has no relation with the incidence of spongy tissue.

Subramanyam et al., (1971) studied the nutritional status of healthy and affected pulp in Alphonso mango. They reported 0.074 % of calcium and 0.12 % of phosphorous in spongy tissue affected pulp as compared to 0.085 % calcium and 0.096 % phosphorous in healthy pulp. Shantha Krishnamukrthy (1981) reported that internal breakdown is not related to the calcium content of the pulp. The concentration of potassium was lower and
phosphorous was higher in the spongy tissue when compared to the healthy pulp. Rangawala (1975) observed that Calcium, Nitrogen, Phosphorous, Potassium and Magnesium contents were higher in the spongy tissue pulp and also in the leaves of affected fruit trees when compared to the pulp and leaves of healthy fruits. But Katrodia (1979) observed that the chemical composition of spongy tissue and healthy tissue surrounding the spongy tissue did not differ significantly in their macro and micro element contents. According to Young et.al.,(1961) the tendency towards soft nose disorder in Kent mango fruits is aggravated by high nitrogen levels in the tree but the high calcium levels in the tree may alleviate this tendency or retard the development of the disorder.

Gunjate et.al.,(1979 a) reported that the calcium was lower in the spongy tissue affected fruits than in the healthy fruits. Calcium content varied at different locations within the fruit. It was more at the upper part and near the skin followed by middle part and central part of the pulp and it was lower at the lower part of the fruit (beak end) and near to the stone. The study indicated that the occurrence of spongy tissue was associated with lower calcium content in the fruit. Maximum spongy tissue was observed at the lower part of the fruit and near to the stone. Burdon et.al.,(1991) studied the mineral distribution in mango cv.Kent susceptible to the physiological disorder softnose. According to their studies minerals were not evenly distributed throughout the mesocarp of mango fruit. The calcium level was highest at the stem end and lowest at the apex of the fruit and the soft nose develops at the apex of the fruit that is at a zone of lowest calcium.

Calcium has long been recognised as an essential element in higher plants (True. 1922). Calcium has received considerable attention because of the close relationship between fruit calcium levels and numerous physiological and pathological disorders. Low levels of calcium have been correlated with physiological disorders of avocados (Chaplin and Scott, 1980), papaya (Quietal 1995), apples (Conway et.al.,1992) and mangoes (Van Eeden 1992).
Calcium treatment has been shown to decrease respiration, reduce ethylene production, delay the onset of ripening and improve fruit quality in apples (Ferguson 1984, Glenn *et al.*, 1990), avocados (Tingwa and Young 1974, Yuen *et al.*, 1990) and mangoes (Tirmazi and Wills 1981, Mootoo 1991, Yuniarti and Suhardi 1992). Calcium can increase the fruit firmness (Bangerth *et al.*, 1972), extend the storage life and can reduce the storage rot (Sharples and Johnson 1977, Poovaiah 1986). Calcium is a normal constituent of the cell wall and middle lamella. Free carboxyl groups on the polygalactouronate polymers play an important role in stabilizing and maintaining cell wall integrity probably through the cooperative binding of calcium ions (Grant *et al.*, 1973). Polyuronides probably arising from the middle lamella (Knee 1984) are responsible for fruit firmness and are liberated during fruit ripening which leads to the breakdown of the aggregation. If the loss of calcium ions is the change which leads to this breakdown then post harvest application of calcium may prevent the dissociation and result in firmness of the fruit.

Post harvest pressure infiltration of 2% or 4% calcium chloride solutions into Golden delicious apples increased both the total and cell wall bound calcium of the fruit and thus increased the fruit firmness (Conway *et al.*, 1995). According to Ferguson *et al.*, 1995, inadequate levels of fruit calcium which lead to disorders such as bitter pit are probably most critical in the extracellular volume of the fruit tissue. Calcium concentrations in the free space fluid may decline during fruit ripening leading to localised deficiency and consequent disruption of cell function, either through the breakdown of the plasma membrane selective permeability or through inactivation of the calcium mediated signaling system. The disorders such as bitter pit may be the physical manifestation of these processes.

Calcium maintains plasma membrane integrity, osmoregulation and extends the post harvest firmness of the fruits. It is also essential for the synthesis of enzymes and the functional macromolecular structure of cellular membranes, microtubules and microfilaments (Evans *et al.*, 1991, Poovaiah 1985).
Calmodulin, a ubiquitous calcium binding protein is involved in many of the calcium dependent changes in plants and calmodulin mediates calcium signals in most of the eukaryotic cells (Cheung 1980, Klee et.al.,1980, Poovaiah et.al.,1987). Low concentrations of Ca\(^{2+}\) salts as pre harvest sprays or post harvest dips have been shown to reduce the bitter pit of apples (Mielke and Fecteau 1988), cork spot of pear and reduce winter freeze damage to pears (Raese 1988, Raese and Drake 1992, 1993), Splitting of sweet and sour cherries (Drake and Proebsting 1985), Anderson et.al.,1989, Campbell and Anderson 1993). Calcium applied as 1 - 3 % CaCl\(_2\) also maintained cellular turgidity in processed sweet and sour cherries (Drake et.al.,1985, Anderson et.al.,1992).

According to Anderson and Campbell (1995) application of pre harvest foliar sprays of sour cherry trees with a solution of Calcium metalosate (an amino acid calcium chelate AACa) and post harvest dips of cherry clusters in AACa and CaCl\(_2\) treatments produced firmer sour cherry fruits and decreased the susceptibility to impact damage. According to Simon (1978) if the fruit is calcium deficient, it may crack or individual cells burst. Calcium deficiency renders membrane permeable so that cell fluids invade the intercellular air spaces of the tissue which leads to dessication in the fruits.

Sampaio et.al.,(1999) studied the effect of foliar calcium sprays on physiological disorders in mango. During fruiting of 8 years old mango cv.Tommy Atkins trees growing in Piracicaba, Brazil, foliar sprays of calcium chloride (0.6 % and 1.2 %) were applied. 7 times at 2 week intervals. Fruits were harvested on 2 dates 16\(^{th}\) December 1996 (normal harvest) and 6\(^{th}\) January 1997 (late harvest). There was no increase in the calcium content in the fruits of treated trees as compared to untreated controls. The incidence of the internal breakdown was similar between treatments, independent of Nitrogen or Calcium contents in the mesocarp. This disorder increased with the later harvest in all treatments.

Gunjate et.al.,(1979 b) reported that the single and double preharvest dips of fruits in calcium solution significantly increased the calcium content in ripe fruits whereas there was no significant increase in calcium content by post harvest calcium dip treatment.
They claimed that preharvest dip treatments significantly reduced the occurrence of spongy tissue in ripe Alphonso fruits and the treatment with calcium chloride was more effective than calcium nitrate. Katrodia (1979), however observed that calcium chloride dip treatments had no effect either on spongy tissue development or its control but the higher concentrations (2 % and 3 %) caused blackening and roughening of the fruit skin. Spraying of shoots with chemicals like Zinc sulphate, Borax and Gibberlic acid or with micronutrients like Zinc, Copper, Iron and Manganeese (in sulphate form) alone or in combination had no significant effect. Shantha krishnamurthy (1982) studied the effect of Calcium and Boron on the incidence of internal breakdown in Alphonso mango.

Drench preharvest sprays of calcium chloride at 5000 ppm and boric acid at 500 ppm either alone or in combination 3 times at monthly intervals were done. And also post harvest dip treatment with 2500 ppm and 5000 ppm calcium chloride was carried out. But both pre harvest and post harvest treatments with calcium did not reduce the incidence of internal breakdown or enhanced the calcium level of the fruits. Haribabu (1993) reported that spongy tissue affected pulp contained very low calcium (19.2 mg/100gm dry weight of pulp) as compared to high calcium (41.7 mg/100gm dry weight of pulp) content in the healthy tissue of the same fruit. But pre harvest calcium sprays of 5000 and 10,000 ppm and post harvest infiltration with calcium chloride had significant effect on the occurrence of spongy tissue. Joyce et.al.,(2001) studied the effect of post harvest calcium infiltration at different stages of maturity in the 4 mango cultivars Kensington, Sensation, Irwin and Palmer varieties. According to the report calcium infiltration at – 33 kpa with 4 % (w/v) calcium chloride did not increased the fruit calcium level in any of the cultivars at any stage of maturity.

Contrary to the above reports Vishwanath (1992) reported that when pre harvest foliar sprays of 4% calcium chloride was given three weeks prior to harvest. The incidence of spongy tissue was reduced by 48 % in comparison to the unsprayed trees. Soil application of 4 kg/tree Dolomite was also found to reduce the spongy tissue. Injections of 500 mg and 1000 mg CaCl$_2$ into the fruit were found to reduce the incidence of spongy tissue by 53 % and 68 % respectively. Chitarra, A.B., et.al.,(2001) studied the
biochemical changes in Tommy Atkins mango fruits stored under refrigeration following pre harvest treatment with calcium chloride. The fruit trees were sprayed with 0 %, 2.5 % and 5 % calcium chloride solution 40, 60 and 90 days after blooming and then the fruits were packed in a ventilated carton and stored at 10°C and 90 % RH for 35 days. Calcium chloride treatment at 5 % resulted in lower mass loss and better texture than the 0 % (control) and 2.5 % treatment. Also the treated fruits did not exhibit breakdown symptoms and were still firm and good for consumption at the end of the storage period (5 weeks). Tirmazi and Wills (1981) reported that the vacuum infiltration of calcium chloride was effective in delaying the ripening of mangoes through no evaluation of internal breakdown or calcium content was made.

Plich. H. et.al.,(2002) studied the effect of Calcium and Boron foliar application on post harvest plum fruit quality. Treatments with Calcium or Boron did not have any impact on the occurrence of internal breakdown during long term storage at low temperature in both cultivars but calcium sprays caused a marked increase of fruit firmness at harvest and slowed the softening during long term storage at low temperature. Wojcik. P. et.al.,(2002) studied the efficiency of different foliar applied calcium materials in improving apple quality. Szampion apple trees were sprayed with Rosatop calcium (22 % calcium as calcium nitrate), Rosafos (4 % calcium as calcium hydrogen phosphate), Rosacal (19 % calcium as calcium nitrate) or calcium chloride (29 % calcium). Calcium sprays reduced the number of apples affected by bitter pit and increases fruit resistance to internal breakdown but had no effect on firmness. Rosatop calcium sprays were more effective in increasing fruit calcium compared to calcium chloride or Rosacal.

2.2.4 Biochemical factors.

(a) pH and acidity: According to Patkar et.al.,(1984) the acidity of healthy fruit was 0.31 %. The acidity was slightly more in unaffected part of affected fruit (0.37 %), but it was maximum in spongy tissue (0.68 %). pH of the healthy pulp was slightly more (4.55) than the pH of unaffected part of the affected fruit (4.48) and pH of the spongy
tissue (3.80). Similar results have also been reported by Subramanyam et al. (1971), Katrodia et al. (1988), Amin (1967) and Rangawala (1975).

(b) Ascorbic acid:— Amin (1967) reported a great reduction in the ascorbic acid content in the affected part. Similar trend of ascorbic acid content in the spongy tissue was found by Chhatpar et al. (1968 - 69). They attributed this reduction in ascorbic acid to the utilization of acid by bacterial isolates. Rangawala (1975) observed the decreasing trends of ascorbic acid content from the healthy ripe pulp to the unaffected part of affected fruit and the spongy tissue. On the contrary, Subramanyam et al. (1971) observed high ascorbic acid content in the spongy tissue as compared to healthy tissue of Alphonso mango.

Katrodia (1979) observed ascorbic acid to be very much reduced in the spongy tissue (13 mg/100 gm) as compared to that in the healthy pulp (72.5 mg/100gm) and unripe pulp (205 mg/100 gm). He reported that much of ascorbic in affected tissue was oxidized at high temperature of fruit pulp.

(c) Beta carotene:— Chhatpar et al. (1968-69), Subramanyam et al. (1971), Rangawala (1975) and Patkar et al. (1984) have observed significant decrease in carotenoid pigments in spongy tissue as compared to healthy tissue in the ripe Alphonso fruits. Katrodia (1979) also reported that there was high reduction of beta carotene in spongy tissue (1501 μgm/100gm) as compared to healthy pulp (5968 μgm/100gm) and unripe pulp (1650 μgm/100gm). He claimed that the reason for the reduction of betacarotene in spongy tissue may be due to its lesser production or its degradation probably due to high temperature in the fruit pulp because of convective heat received by the fruit on tree.

(d) Starch Content:— Rangawala (1975) observed 1.47 % starch in spongy tissue affected part of ripe fruit as against no starch in healthy ripe pulp. According to Patkar (1984) starch was completely absent in healthy ripe fruits, negligible in the unaffected part of the affected fruit but considerable amount of starch was found in the spongy tissue.
(8.25 %) which may be due to decreased amylase activity that might have resulted in the reduced hydrolysis of starch into sugars in the spongy tissue. Chhatpar et.al.,(1968-69) also reported the decreased amylase activity in the spongy tissue.

Katrodia (1979) observed that much of the starch in the affected tissue remained unhydrolyzed and it was 7.98 % in spongy tissue, 0.25 % in healthy pulp and 12.87 % in mature fruit. Nuevo et.al.,(1984a) reported that the parenchyma cells of internal breakdown affected mango tissues contained an average of 18 starch granules per cell in contrast to only 2 granules in the cells of adjacent healthy tissues which indicates that the starch metabolism has been affected in the spongy tissue.

Katrodia et.al.,(1988) reported that the healthy pulp showed amylase activity as high as 3.21 while it is very much decreased to the extent of 0.39 in spongy tissue and 0.50 in sundesiccated tissue. The lower rate of amylase activities in both the types of affected tissues seems to be the result of high temperature effect in the pulp during ripening. Also Lima et.al.,(2001) reported lower amylase activity and much higher starch content in the spongy tissue of mango cv.Tommy Atkins stored at 12± 2°C and 90 ± 5 % RH for 28 days. Similar trend was reported by Conn and Stupf (1971)

(e) Sugars:- Amin (1967) reported appreciable reduction of non reducing sugars in the affected and unaffected parts of spongy tissue affected ripe fruits as compared to healthy one. Similar findings were observed by Chhatpar et.al.,(1968-69), Subramanyam et.al.,(1971) and Rangawala (1975). According to Patkar et.al.,(1984) reducing sugars were slightly more in the spongy tissue (4.47 %) when compared to the unaffected healthy tissue of the affected fruit (3.97 %) and the healthy tissue of the healthy ripe fruit (4.23 %). However, non-reducing sugars were considerably lower in the spongy tissue (4.40 %) when compared to the unaffected part of the affected fruit (11.10 %) and healthy ripe fruit (11.35 %). Lima et.al.,(2001) reported lesser reducing and non reducing sugars in the spongy tissue of Tommy Atkins mango fruits.
According to Katrodia (1988) there was reduction in the reducing sugars in the spongy tissue (3.42 %) when compared to healthy pulp (8.30 %). The invertase activity was also found to be badly affected in the spongy tissue (0.46 U) when compared to the healthy pulp (1.79 U). Because of heat the activities of amylase and invertase were retarded and the normal sugar metabolism was deregulated ultimately the carbohydrates were found degraded. Contrary to this higher invertase activity was observed in the spongy tissue (Patkar et al., 1984, Subramanyam et al., 1971 and Gupta et al., 1985). Also the spongy tissue showed lesser contents of total soluble sugars (TSS) and higher phenolic contents as compared to healthy tissue. (Subramanyam et al., 1971, Patkar 1984).

The affected mango cv. Alphonso and Mallika were separated into spongy tissue, healthy tissue and healthy surrounding spongy tissue and were biochemically analysed by Selvaraj et al., (2000). They reported that the breakdown tissue had low carotene, sugars, sucrose, sugar to acid ratio, potassium, calcium, sodium and higher acidity, glucose to fructose ratio, Nitrogen, Phosphorous, Magnesium, Zinc and Iron contents as compared to healthy tissue. Also the breakdown tissue had twice the amount of citric acid and half the quantity of malic acid as compared to healthy tissue. Glycolytic enzymes, hexokinase, pyruvate kinase and phospho fructokinase and gluconeogenic enzymes Fructose bis phosphatase and phospho enol pyruvate carboxy kinase activities were low. Also the fruit softening enzyme activities (pectin esterase and β-galactosidase) were affected in the breakdown tissue.

A study was carried out by Lima et al., (2000) on the Tommy Atkins mango fruits harvested at physiological maturity and stored at 12°C for upto 28 days. They reported that the affected spongy tissue had lower concentrations of ascorbic acid, soluble pectin and lower activities of polygalactouronase and pectin methyl esterase. Higher activities of peroxidase, polyphenyl oxidase and phenylalanine ammonia lyase were observed in the spongy tissue affected Tommy Atkins mango fruits stored at 12°C, 90 % RH for 28 days (Lima et al., 1999). Chitarra et al., (1999) also reported that the fruit with internal breakdown had higher cell wall polyuronides content than the healthy ones due to
inhibition of pectin hydrolases activity in Tommy Atkins mango fruits. According to Shantha krishnamurthy (1981) the spongy tissue of affected Alphonso mango fruit had higher concentrations of pyruvic acid and α-ketoglutaric acid. The activities of malic enzyme and pectin methyl esterase were also higher in the breakdown pulp than in the healthy pulp. Accumulation of acetaldehyde was observed in the spongy tissue.

Nuevo et.al.,(1984) reported that the onset of internal breakdown appears to be induced by oxygen starvation with a distinct fermented odour in the severely affected fruits. Serrano,(unpublished) showed that under low oxygen conditions ethanol and acetaldehyde levels increases in the ‘Carabao’ mango fruits. Gupta et.al.,(1985) studied the various enzyme activities in the spongy tissue affected Alphonso mango fruits. According to the report the spongy tissue exhibited about sixteen times lesser amylase activity and 2.5 times higher invertase activity as compared to healthy pulp. The activity of ascorbic acid oxidase was seven times higher in the spongy tissue as compared to healthy pulp. However the activity of both peroxidase and catalase was found to be much less in the spongy tissue when compared to the healthy tissue.

Also the spongy tissue exhibited much lower activity of glutamate dehydrogenase (GDH) and negligible activity of glutamate oxaloacetate transaminase (GOT). GDH and GOT enzymes have been shown to play an important role in the amino acid biosynthesis in the ripening fruits. The decreased protein content in the spongy tissue may be due to the rapid breakdown of protein or inhibition of protein synthesis. The phenol levels were very high in spongy tissue which are known to exert inhibitory effects on several enzymes. Active oxygen species are generated during several plant processes. Mitochondrial electron transport chain at high temperature is one among them. Reactive oxygen radicals are cytotoxic which can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in the plasmalemma or intracellular organelles. Peroxidation damage of the plasmalemma leads to leakage of cellular contents, rapid desiccation and cell death (Henley and Linn, 1997, John. G. Scandalios, 1993).
According to Kumar et al. (1990) increase in temperature during the initial stages of ripening in mango may result in generation of active oxygen species and could be one of the causative factors for formation of spongy tissue. An increase in the activities of enzymes like peroxidase and catalase in mango during ripening also indicated that there was higher generation of free radicals during ripening. (Day et al., 1980, Keista et al., 1998 and Mattoo et al., 1968). Gupta et al., (1985) reported lower activities of both catalase and peroxidase in spongy tissue.

Iron content is more in mango fruit when compared to other fruits. Reduced activities of catalase and peroxidase coupled with excess iron, peroxide radicals are known to enhance the oxidative damage of the membranes. Also reduction in the activities of free radical scavenging enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase during the initiation of ripening was reported to be the reason for sunscald in tomatoes (Rabinowitch, H.D and Sklan, 1980). Activities of SOD and CAT were found to be related to the evolution of ethylene in apple (Masia, 1998). Free radical scavenging enzymes and ethylene are important in maintaining the membrane integrity and tissue texture in fruits.

2.3. Possible control measures for the disorder.

Although the incidence of spongy tissue was reported as early as 1932, not much is known regarding its exact cause and control. Numerous factors are known to influence the incidence and extent of infection, but the primary causal factors has remained elusive (Katrodia et al., 1988, Wainwright and Burbage 1989). Therefore an integrated approach should be done to control certain pre and post harvest factors for reducing the occurence of spongy tissue. The single and double pre harvest dips of fruits in 0.5 % and 2 % calcium chloride and calcium nitrate solutions significantly reduced the occurence of spongy tissue in ripe Alphonso fruits (Gunjate et al., 1979). Soil application of 4 kg/tree of Dolomite was also found to reduce the spongy tissue. And injections of 500mg and 1000mg calcium chloride solutions into the fruits also reduced the incidence of spongy tissue by 53 % and 68 % respectively (Vishwanth et al., 1992).
Avoiding heavy doses of Nitrogen containing fertilizers also found to reduce the incidence of softnose in kent mangoes (Young et al., 1961). Sod culture having natural vegetation of Darbh (Eragrostis cynosuroides L) could control the occurrence of spongy tissue in the fruits to the extent of 100 percent (Katrodia et al., 1988). Green vegetation or mulching also reduces the disorder at pre harvest stage by keeping the soil surface temperature down (Katrodia et al., 1988).

Post harvest protection of fruits from exposure to sunlight, use of ventilated corrugated fibre board (CFB) boxes and use of tissue paper for cushioning of fruits, transportation of fruits during cool night time are supposed to reduce the incidence of spongy tissue in mango fruits. (Gunjate 1982, Lad et al., 1992). Post harvest dipping of mango fruits in 500 ppm, 750 ppm and 1000 ppm solution of ethephon could reduce the occurrence of spongy tissue in Alphonso mango (Lad et al., 1985). This disorder can possibly overcome by using resistant varieties in the breeding programmes (Iyer and Subramanyam, 1992). In 1976, the cultivar Alphonso, which is susceptible to spongy tissue was used as a common parent and crossed with Banganpalli, Neelum, Kalapady and Janardhan pasand which are free from the ripening disorder.

Evaluation of progeny showed that spongy tissue formation is genetically controlled. The character is monogenic and susceptibility to the disorder is recessive, with Alphonso being homozygous recessive. Screening of the hybrid progenies enabled the isolation of two promising hybrids: Hybrid 10 and Hybrid 13 resembled Alphonso in terms of fruit colour, size, quality and flavour. Also they were free of the disorder. Some of the recently developed mango hybrids ‘Ratna’ (Salvi 1983), Sindhu (Gunjate and Burondkar 1993) and Arka Puneet (Iyer and Subramanyam 1993) were found to be resistant to this disorder may be attributed to the genetic make up of the hybrid.
References.


Gunjate, R.T., Tare, S.J., Rangawala, A.D and Limaye, V.P., 1979 b. Effect of preharvest and post harvest calcium treatments on calcium content and occurrence of spongy tissue in Alphonso mango. *Ind. J. Hort.*, 36: 140-144.


Gupta, D.N., LAD, B.L., Chavan, A.S., and salvi, M.J., 1985. Enzyme studies on spongy


Joshi, G.D., 1975 studies on spongy tissue of mango fruits. MSc. (Agri) thesis submitted to konkan krishi vidya peeth. Dapoli, India.


