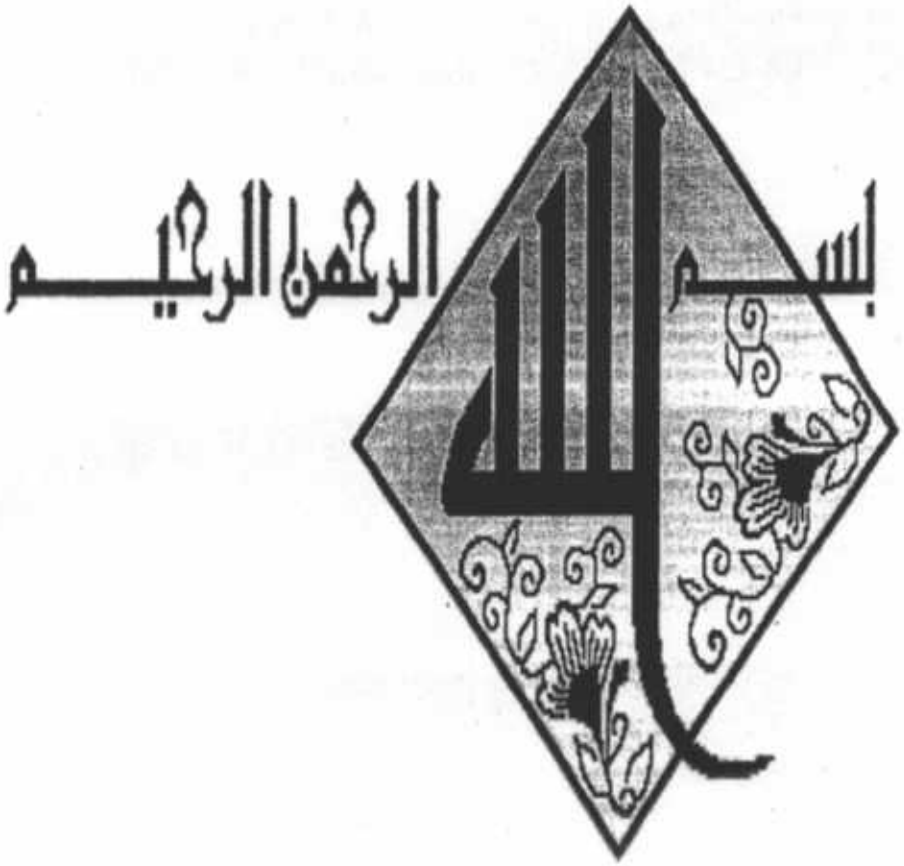

In The Name Of Allah
The Most Gracious, The Most Merciful

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STUDIES ON THE AMYLASES OF MANGO FRUIT

BY

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M.Sc. (Hons.) Food Technology

A thesis submitted in partial fulfilment of
requirements for the degree of
DOCTOR OF PHILOSOPHY
IN
FOOD TECHNOLOGY
FACULTY OF AGRICULTURAL ENGINEERING AND TECHNOLOGY
UNIVERSITY OF AGRICULTURE
FAISALABAD

1978

ACKNOWLEDGEMENTS

The author expresses his deep gratitude to Dr. Haji Muhammad, Professor, Department of Nutrition, for his invaluable and inspiring guidance and help in the successful conduct of research and writing of this manuscript. He is indebted to him for his valuable suggestions, constructive criticism and keen interest.

The author is also highly grateful to Dr. Muhammad Shafiq Chaudhry, Chairman, Department of Food Technology, Dr. Ali Asghar, Associate Professor, Department of Food Technology and Dr. Syed Abrar Hussain Gilani, Associate Professor, Department of Physiology, for their kind help from time to time throughout the course of this research work.

Thanks are also due to Ch. Mansoor Ullah, ex-Food Technologist, Punjab Agricultural Research Institute Faisalabad, for providing research facilities.

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CHAPTER I

INTRODUCTION

Mango (Mangifera Indica L.) is one of the oldest fruits and has been cultivated by man for over 4000 years, originating apparently in the Indo-Pak region. It is, at the present time, the most popular fruit among millions of people in the orient where it is appreciated as the choicest of indigenous fruits, occupying, according to Singh (1960), relatively the same position in the tropics as the apple in Europe and North America. With a view to expand the local and world trade of mango increased emphasis has recently been placed on its export as fresh fruit. Its preservation in fresh state for extended periods without loss of quality is, therefore, a problem that merits further studies.

For the preservation of fresh fruits, the attempt has to be made to arrest or at least retard the changes associated with the climacteric and post-climacteric phases. The metabolic changes in fruits are known to be affected to a great extent by such factors as temperature, surrounding gaseous atmosphere, irradiation and chemical treatments. However, it seems that no direct approach has been made to control or shift a particular type of change associated with the ripening of mango fruit. The disappearance of starch is one of the most dramatic chemical changes associated with ripening of mango fruit. Enzymatic hydrolysis of starch is performed by amylases. The primary forms of amylases are denoted as alpha and beta. α-amylase hydrolyses the α-1,4-glucosidic linkages randomly whereas β- amylase hydrolyses alternate α-1,4

glucosidic bonds systematically starting from the non-reducing end of the molecule resulting in maltose formation. Neither α-nor β-amylase is capable of transversing or hydrolysing the α-1,6-glucosidic bond at the branching points of the amylopectin fraction. This bond is broken by isoamylase-an α-1,6- glucosidase (Hulme, 1960). The increase in amylase activity and dramatic disappearance of starch associated with the ripening of mango fruit, if controlled might help in the shelf life extension for longer period of time. So an understanding of mango fruit amylase was considered necessary and studies were planned keeping in view the following objectives:-

1. To extract and determine the activity of mango fruit amylases as influenced by pH and temperature conditions.
2. To observe the effect of pH and temperature on the activity of amylases and disappearance of starch during storage of preclimacteric mango fruit slices.
3. To study the chemical and biochemical changes associated with the mango fruit ripening during various fruit development stages, fruit ripeness states and to also observe the ripening behaviour of the fruit at various storage temperatures with a view to achieve extended shelf life of the fruit.

CHAPTER II

REVIEW OF LITERATURE

For a systematic review of the previous research work on different aspects, the relavant information has been treated under the following headings:-

1. Model system for enzyme studies.
2. Amylase activities during development, ripening and storage of fruit.
3. Enzyme extraction problems.
4. Biochemical changes associated with the development and ripening of mango fruit.
5. Effect of external factors on the ripening behaviour of mango fruit.

1. Model system for enzyme studies

The study of biochemical changes occurring during the ripening phase in fruits would be greatly simplified if pieces of tissues rather than whole fruit could be used. Rhodes et al. (1968) found that many of the biochemical changes in ripening apples also occurred in discs of preclimacteric apple peel tissues which were aged for 24-48 hours in buffer solutions containing chloroamphenicol. The concentration of the chloroamphenicol used to inhibit the action of any microorganism present were reported to have no effect on the metabolism of the fruit tissues. From these facts and further series of experiments carried out by Rhodes et al.(1968) with aged preclimacteric apple peel, it was concluded that aging fruit

discs could provide a model system for detailed study of at-least some of the enzymic processes occurring during the respiratory climacteric of whole fruit.

2. Amylase activities during development, ripening and storage of fruits

McArthur-Hespe (1956) studied amylase activity in cortical tissue of several varieties of pear during growth, maturation, ripening and storage. Pear exhibited an increase (per fruit basis) in starch hydrolytic activity during development. The most significant increase in activity occurred during the months preceding commercial harvest maturity and not earlier. She further reported that this activity continued to increase in the fruit even during storage for 3 months at 0.5°c, but not during ripening after removal from storage. She related this fact to the failure of the fruit to ripen as normal. Less mature fruit of Comptess-de-Paris variety underwent a greater increase in starch hydrolytic activity than that of late picked fruits which exhibited capability of ripening even after much longer storage period.

An increase in amylase activity in mango fruit during ripening was observed by Mattoo and Modi (1969) whereas during cold storage of mango fruit Chatpar et al.(1971) reported a decrease in amylase activity on account of the development of chilling injury. Mattoo and Modi (1970) extracted an amylase inhibiter from unripe mango and attempted its 100-fold purification.

3. Enzyme extraction problems

For isolating active enzyme from the fruit apparently two

problems are encountered. The first seems to be the high acidity of the vacuolar contents of the fruit cells. If the fruit acidity is not neutralized, it would come in contact with the proteins of the cells, during maceration and enzyme extraction, resulting there-by in protein denaturation and its deposition on the cell debris. This difficulty could however be overcome either by the use of mildly alkaline buffers as extraction media or by the gradual addition of alkali during maceration of tissue (Hulme, 1946). The second problem being due to the high phenolic (tannin) contents of the fruit. When not properly arrested, the phenolic compounds would become tightly bound to the protein fractions of the cells, during maceration and enzyme extraction, causing reduction or entire inhibition in enzyme activity. Luckily this problem has been overcome by the use of polyvinylpyrrolidone (PVP), which rapidly binds phenols by hydrogen bonding (Jones et al; 1965) in the extraction medium.

4. Biochemical changes associated with the development and ripening of mango fruit

Singh et al. (1937), Leley et al. (1943) and Mukerjee (1959) while studying the physiological changes occurring during growth and development of mango fruit, observed that the fruit developed to its full size in 90 days and the physical size and weight remained constant thereafter. Regarding chemical changes, Lakshimarayana et al. (1970) has reported that starch accumulated throughout the period of development and maturation, but acidity decreased progressively after reaching its peack around the 7th week of development. Mango fruit maturity has been reported to be closely

associated with the starch content and with the specific gravity of the fruit in six varieties of Florida mangoes (Propenoe and Long; 1957).

According to the common practice, mangoes are seldom allowed to ripen on the trees. These are usually picked at full maturity prior to the onset of ripening. The process of ripening is completed during subsequent storage. Ripening has been shown to be accompanied by many chemical and biochemical changes in the fruit. Several studies regarding the changes in sugar, starch and acidity during ripening of mangoes have been reported. The studies by Soule and Harding (1956), Wali and Hassan (1965), DeLon and DeLima (1968), Krishnamurthy et al. (1971), and Elahi and Khan (1973) have indicated the complete hydrolysis of the starch and several fold decrease in acidity during ripening of mango fruit. The quality of the ripened fruit has been observed by Harding et al. (1954) to be closely associated with moderately higher percentage of total solids and total acids of the fruit at harvest.

5. Effect of external factors on the ripening of mango fruit

Ripening rate and storage quality of mango fruit were dependent upon the storage temperature (Mukherjee, 1958). According to the findings of Holz et al. (1971), the best storage temperature for the mangoes was 13°c, since the fruit could be kept undamaged for two to three weeks, whereas at temperature below 10°c these were susceptible to chilling injury.

Extension in shelf life of mango could also be achieved through controlled atmosphere storage. Kapur et al. (1962) have

shown that Alphonso and Pairi mango varieties could be kept under refrigerated gas storage for 35 and 49 days at 8.3 to 10°C and 5.5 to 7.2°C respectively within a 10 per cent wastage level.

According to Dharkar et al. (1966), and Dennison and Ahmed (1967) the ripening process of mangoes could be delayed through irradiation by γ-rays. A combination of skin coating with wax emulsion and irradiation proved more effective in extending the storage life of mangoes.

Post-harvest treatment of mango with the maleic hydrazide and 2,4,5 trichlorophenoxypropionic acid resulted in delayed ripening process without altering the quality of the fruit (Krishnamurthy and Subramanyam, 1970). The use of ethylene dioxide has been reported by Subramanyam et al. (1969) as most effective fumigant to control spoilage and inhibit ripening of mango fruit.

CHAPTER III

MATERIALS AND METHODS

Fruit samples

Mango fruit of Dusehri variety was picked at various stages of development from a group of four trees. These trees were approximately of the same age, growth and spread and were located in the experimental garden of the Punjab Agricultural Research Institute, Faisalabad. The fruit was transported to the laboratory within two hours after harvest, where it was graded for uniformity in size, colour and weight. Only medium sized fruits of the same colour and weight were selected for this study.

Preparation of enzyme extract

Enzyme extracts were prepared by blending 100 gm of mango fruit pulp with 50 ml distilled water containing 0.1 per cent polyvinylpyrolidone for inhibiting the action of tannins (Jones et al. 1965). The pH value of the blend was maintained at about 7.0 with 0.2 M carbonate-bicarbonate buffer (pH 9.2) to avoid precipitation of enzyme particularly due to low pH value of the unripe fruit (Hulme, 1946). The clear supernatent was obtained after centrifugation under refrigerated atmosphere at 608 x g for 10 minutes. All the above operations were carried out at a temperature of 5^{0}C or below. The extracts thus obtained were preserved with a few drops of toluene and stored in refrigerator. Stored enzyme extracts were subsequently utilised for partial purification of amylases and determination of their activity.