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TO EVALUATE THE EFFECT OF ETHANOL INJECTION TREATMENT IN DELAYING RIPENING OF MANGO (*Mangifera indica)*

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ABSTRACT

India is the second largest producer of fruits & vegetables and is the leading producer of mango in the world; yet post-harvest losses continue to be tremendous in India. Ethanol is known not only to reduce ethylene production but also its action. This research work was conducted to evaluate the effect of ethanol treatment in enhancing the shelf life of mango. It has been concluded that ethanol treatment of mature unripe mango fruits enhanced their shelf life by 7 days in comparison to control. Ethanol treatment was found to delay chlorophyll degradation, carotenoids biosynthesis and weight loss. Experiments were conducted to monitor the change in fruit weight, peel color, total chlorophyll content, carotenoid content, titrable acidity and starch content.

Keywords: Mango, Ethanol treatment, Acetaldehyde, Ethylene, Climacteric fruit ripening

INTRODUCTION

Mango, also known as king of fruits, is a tropical tree cultivated in many different regions around the world and India. It is one of the most popular fruit with unique aroma, flavor, taste and health promoting qualities. It is a seasonal fruit with 5-15cm length and 4-10cm width. Mango season in India lasts from April –August .

Mango is rich in dietary fibers, vitamins, minerals and poly flavonoid compounds. Recent studies about mango revealed its role in prevention of several types of cancer. It is a rich source of vitamin A which helps in maintaining good eyesight and healthy skin. It is also a good source of vitamin B6, Vitamin C, Vitamin E, potassium and trace amounts of copper[Rymbai et al., 2016].

There are several causes of post-harvest losses in mango which is affecting country's economy. Ethylene plays a crucial role in triggering ripening of climacteric fruits and therefore, controlling its effect to avoid post-harvest losses is a major challenge for scientists. Some of the important sites where post-harvest losses are noticed in India are —farmer's field, packaging, transportation and marketing.

This research work was conducted to evaluate the effect of ethanol on ripening of mature unripe mangoes in comparison to untreated control mango fruits. Ethanol is known not only to reduce ethylene production but also delays its action.

Production of Mango in India and the world

Table 1 : List of top mango producing countries in the world in 2014

COUNTRY	Production(In Tons) in 2014
INDIA	15,188,000
CHINA	4,350,000
THAILAND	2,500,000
INDONESIA	2,131,139
PAKISTAN	1,888,449

Mango is grown almost in all the states of India. Uttar Pradesh tops the list of mango producing states.

Table 2: State wise production of mangoes in India

S.No.	STATE	Production(million hectares) 2015-2016
1	Uttar Pradesh	4611.2
2	Andhra Pradesh	3049.93
3	Telangana	1797.8
4	Tamil Nadu	1660
5	Karnataka	1652.5
6	Gujrat	1301
7	Orissa	723.13
8	West Bengal	693.39
9	Maharashtra	522.87
10	Jharkhand	414.39

Role of ethylene in fruit ripening

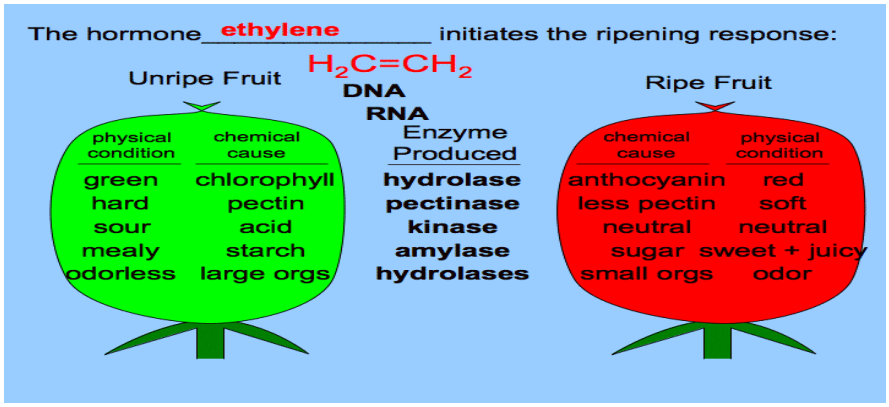


Figure 1: Role of ethylene in ripening of climacteric fruits [Koning et al., 1994**]**

Ripening is the final stage of fruit development and is a co-ordinated process of biochemical differentiation leading to enhanced ethylene production and other associated physiological and biochemical changes. Ripening converts a fruit to an edible state after changes in the physiochemical attributes [Agar et al., 1999].

Fruit ripening is the result of the hormonal signal . The hormone responsible to carry this signal is the biosynthesized ethylene. It is produced throughout the plant's life by all parts of the plants and is regulated throughout the phases of its growth. At the time of ripening (and at normal synthesis), ethylene is synthesized by a complex process of converting amino acid methionine with the help of various enzymes.

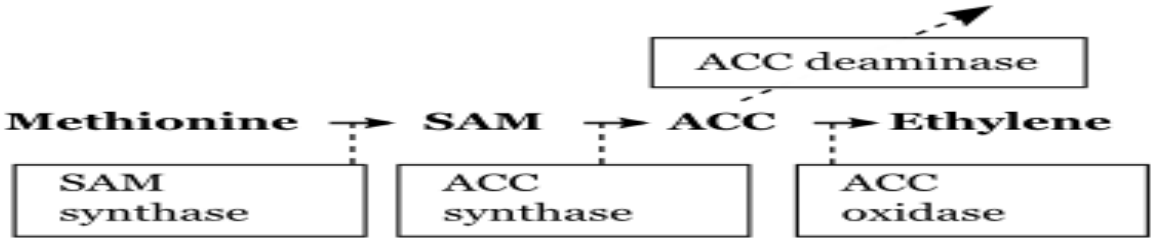


Figure 2: The pathway of ethylene biosynthesis in plants.

The two genes controlling the committed steps to ethylene biosynthesis, ACC synthase (*ACS*) and ACC oxidase (*ACO*) are highly transcriptionally regulated. Ethylene production is closely associated with mitigation of fruit ripening in climacteric fruits, and is the plant hormone that regulates and coordinates the different aspects of the ripening process; color development, aroma production and texture. Typically, fruit generate barely detectable amounts of ethylene until ripening, when there is a burst in its production. This is known as the climacteric rise.

Ethylene production also increases sharply to a maximum at this time, and then declines before fruit ripening. The major rise in ethylene production may take place before, just after or close to the respiratory peak [Oeitkar et al.,1995]. Such fruit are classed as 'climacteric', with apple, avocado, banana, fig, mango, papaya, passion fruit, pear and tomato being classic examples.

Biochemical & Physiological changes during ripening

Taste

The taste of the fruit changes when it ripens. At the initial stage, the fruit is a little tart or sour due to the presence of acids. When the fruit ripens, kinase enzymes turn the acidic fruit to a neutral one by consuming the acids and thereby reducing the acid content. The fruit turns sweet when it ripens, because of the enzyme amylase that converts all the starch present in the fruit to sugars as it ripens.

Color

The color of the fruit changes from green to yellow as they ripen. The color has a significant effect on attracting animals to help the plant in seed dispersal. The yellow carotenoid pigments are synthesized and the chlorophyll is broken down by hydrolase enzymes.

Odor

Hydrolases are also responsible for converting large molecules into smaller aromatic compounds. Aroma characteristic of different fruits also helps in attracting animals, which helps in seed dispersion later.

Hardness

Unripe fruits have usually hard texture. This hardness is due to the presence of pectin in the primary cell wall. The pectin is broken down by pectinase and pectin esterase enzymes, converting the fruit softer and edible while it ripens.

All biochemical and physiological changes during fruit ripening are driven by the coordinated expression of fruit ripening-related genes. These genes encode enzymes that participate directly in biochemical and physiological processes. Generally, the ethylene production within the fruit activates many other enzymes resulting in physiological changes such as the change of color from green to yellow and the softening of the fruit. Excessive softening is associated with an increased expression of cell wall degrading enzymes acting upon protein and carbohydrates such as polygalacturonase, pectin methyl esterase, -galactosidase, and -glucanase[Osorio et al.,2013].

Role of ethanol on climacteric fruit ripening

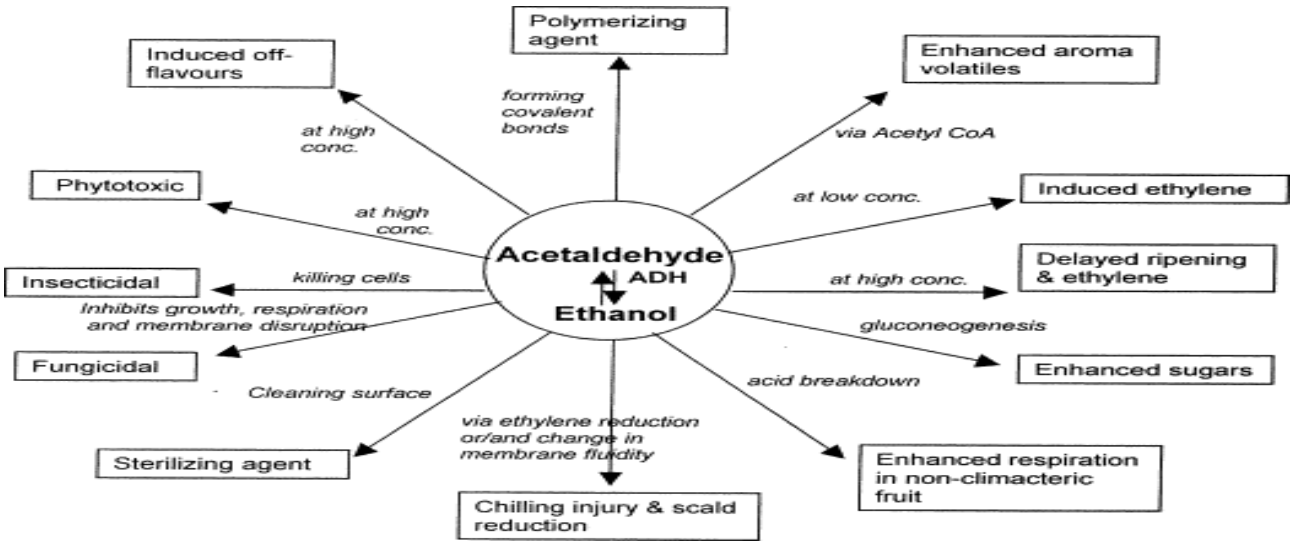


Figure 3: Conversion of ethanol to acetaldehyde by ADH and its effects [Edna Pesis,2009.]

It has been proposed and demonstrated that ethanol-mediated delay in ripening is basically caused by acetaldehyde .Since acetaldehyde, and not ethanol, is the active component in delaying ripening, the conversion of ethanol to acetaldehyde by the tissue is the critical factor in determining whether a certain level of ethanol exposure delays ripening. Acetaldehyde is produced by the conversion of ethanol into acetaldehyde *via* the reversible reaction catalyzed by the enzyme ADH (Alcohol dehydrogenase). Acetaldehyde and ethanol are inter-convertible volatile compounds so they are being discussed together not only in relation to one another but also in terms of their final effect on fruit ripening.

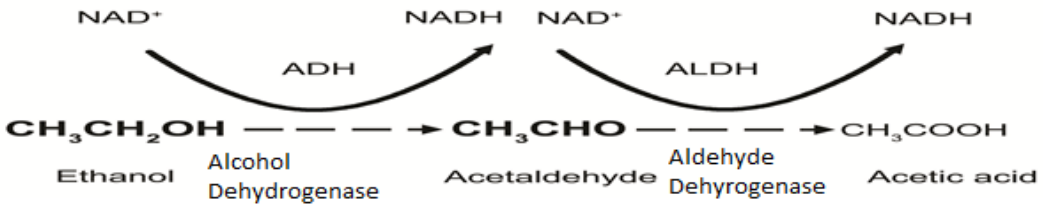


Figure 4: Ethanol to Acetaldehyde conversion [Robert zbeda, 2009.]

Acetaldehyde inhibits the formation of ethylene by preventing the action of ACC-synthase and action and synthesis of ACC-oxidase. Exogenous ethanol application has been earlier reported to cause marked increase in acetaldehyde levels, which inhibits the ethylene production and ripening of mangoes [Pesis et al., 1984].

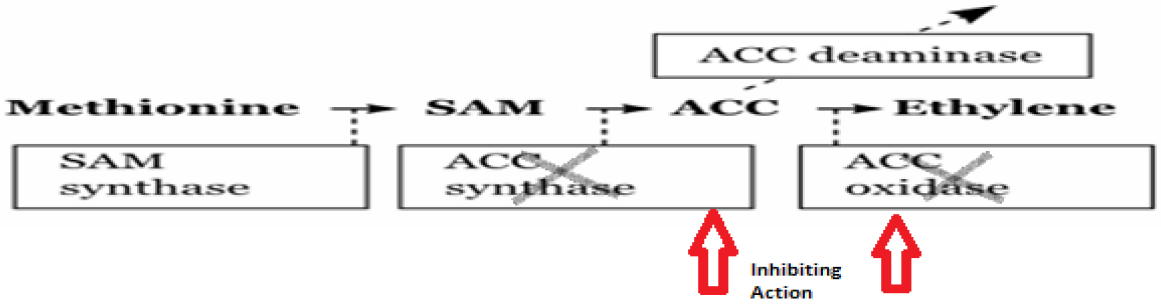


Figure 5: Effect of Acetaldehyde in inhibiting ACC synthase and ACC oxidase

MATERIALS AND METHODS

Unripe mangoes (*Mangifera indica*) variety Dusheri was collected from a local market of New Delhi. Fruits brought to the laboratory were washed and air dried before ethanol treatment.

0.4 ml per 100gm fruit dosage of laboratory ethanol under normal room conditions was given to half the total number of total mangoes and air dried [M.ARitenour et al., 1997]. Individual fruits were weight and their peel color was recorded. Reagent- grade ethanol (95%) was injected into mangoes up to 0.4 ml or in a ratio of 6 ml/kg in the center of the fruit. Control and treated fruits were kept at room temperature for observation on different parameters of ripening.

Figure 6: Ethanol injection given to unripe mangoes using syringe

VISUAL ANALYSIS

Fresh weight

Mango fruits collected were around 100-150 grams in weight .On daily basis, change in fruit weight was recorded with the help of weighing balance and % fruit weight loss was calculated.

Calculation

Fruit weight loss (%) = ((initial weight-final weight/initial weight))*10

Color

Dark green colored mature unripe mangoes were collected from the market. On daily basis, the change in color was recorded with the help of mango color chart.

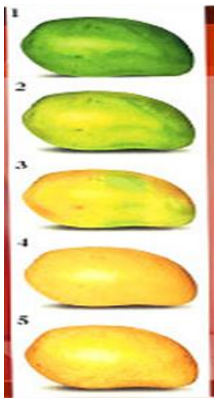


Figure 7: Mango color chart

BIOCHEMICAL ANALYSIS

Estimation of chlorophyll

3g of fruit peel was grounded in 15 ml absolute ethanol followed by centrifugation at 10,000rpm for 20 minutes. After centrifugation, supernatant was divided into 4 equal volumes in different tubes. Finally absorbance was taken at 480nm, 645nm with absolute ethanol taken as blank.

Calculations

Arnon's equation was used to convert absorbance measurements as mg chlorophyll per gram of fruit tissue

a. Chlorophyll a concentration(mg/g):12.7*A663-2.69*A645

b. Chlorophyll b concentration(mg/g):22.9*A645-4.68*A663

c. Total chlorophyll= chlorophyll a + chlorophyll b

d. Carotenoids concentration (mg/g):

$$[A480 + (0.114*A663)-(0.638*A645)]+112.5$$

Iodine test

Iodine solution was poured in a shallow tray. Mango fruit was cut in 2 halves. Fruits were then kept in tray with cut surface dipped in iodine solution. Finally fruits were taken out of the tray after 2-3 minutes and observations on starch in fruits were taken

Titrable acidity

10ml fruit juice was extracted and filtered through muslin cloth. 50 ml of distilled water was added. Simultaneously 2 drops of phenolphthalein was added to diluted fruit juice. Titration was

carried out in presence of 0.1 N NaOH to obtain end point to pink. Readings on volume of NaOH consumed to achieve end point were recorded.

CALCULATIONS

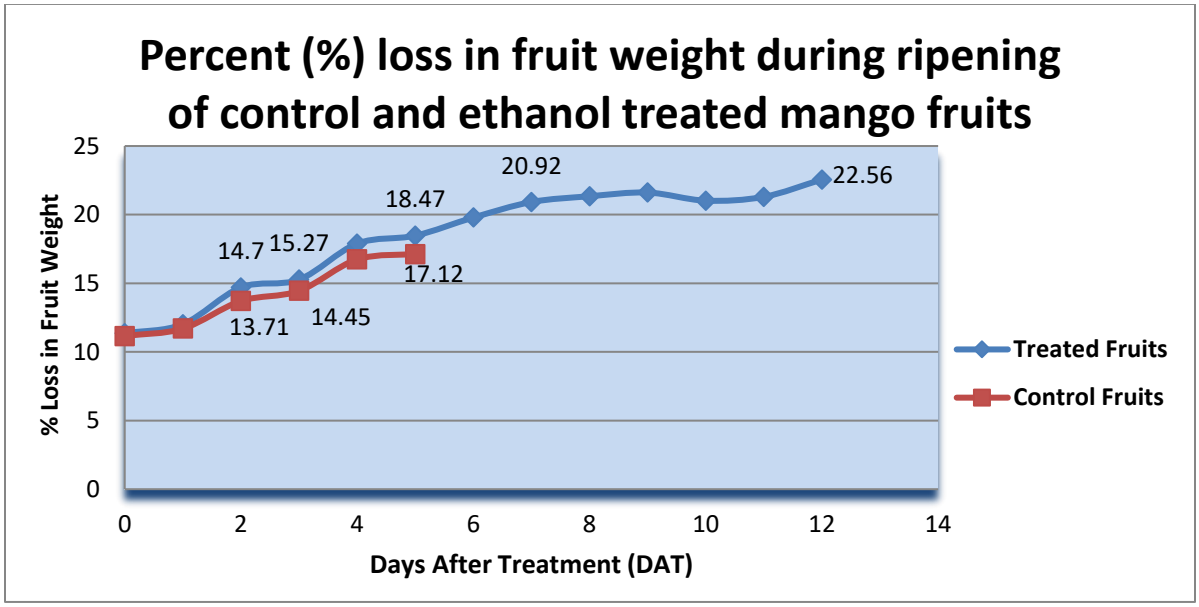
%Acid= volume of NaOH*0.1*miliequivalent factor*100/grams of sample

Acid predominant in mango= Tartaric acid

Grams of fruit sample used= 6 grams

RESULTS AND DISCUSSION

1) **Change in fruit weight**



Graph 1: Percent (%) loss in fruit weight during ripening of control and ethanol treated mango fruits

Loss in weight largely occurs due to respiratory consumption of food reservoirs during ripening [McCarthy et al., 1999]. A significant decline in percent fruit weight loss was observed in control mangoes on 5th DAT in comparison to ethanol treated fruits. On the other hand, ethanol treated mangoes were able to retain their weight till 12th DAT which was comparable to control fruits on the 5th DAT (as shown in Graph 1).Also effect of ethanol dosage on softening was observed, control mangoes were more softer than ethanol treated mangoes on 5th DAT. Activity of cell wall hydrolyses seems to be delayed by ethanol. And since the percent weight loss of

ethanol treated fruits was less in comparison to control it may be concluded that ethanol has delaying effect on respiratory pathway during ripening.

2) Visual analysis

Change in fruit peel color during ripening of control and ethanol treated mango fruits

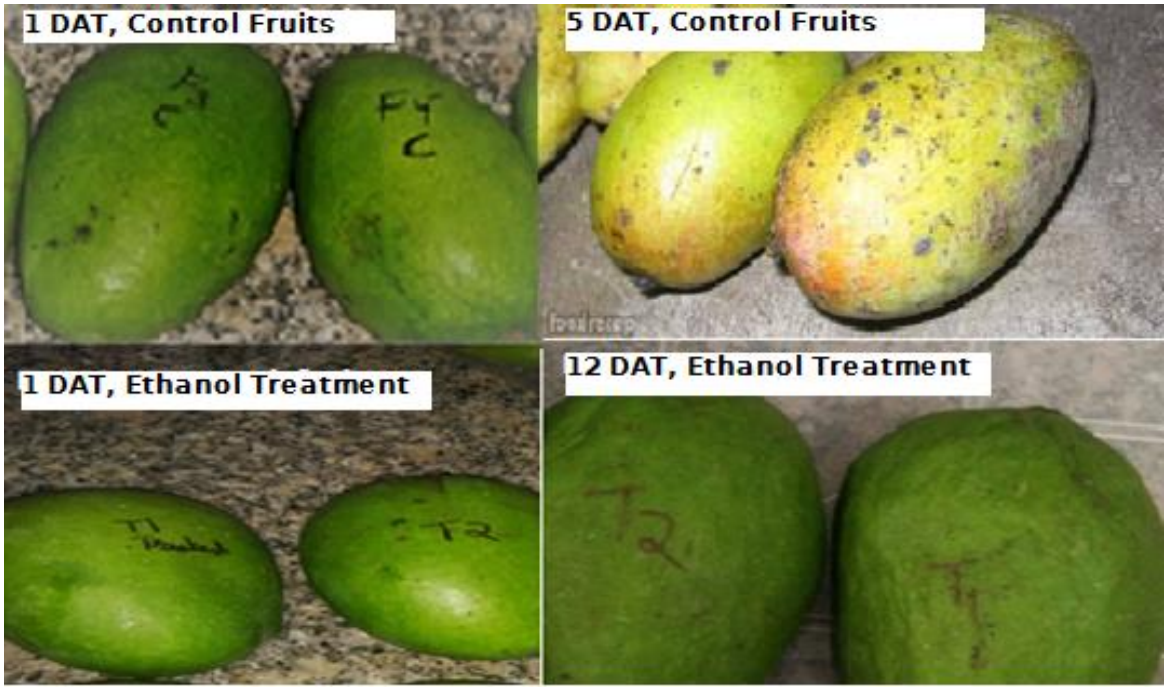
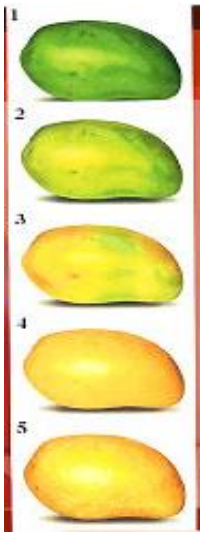
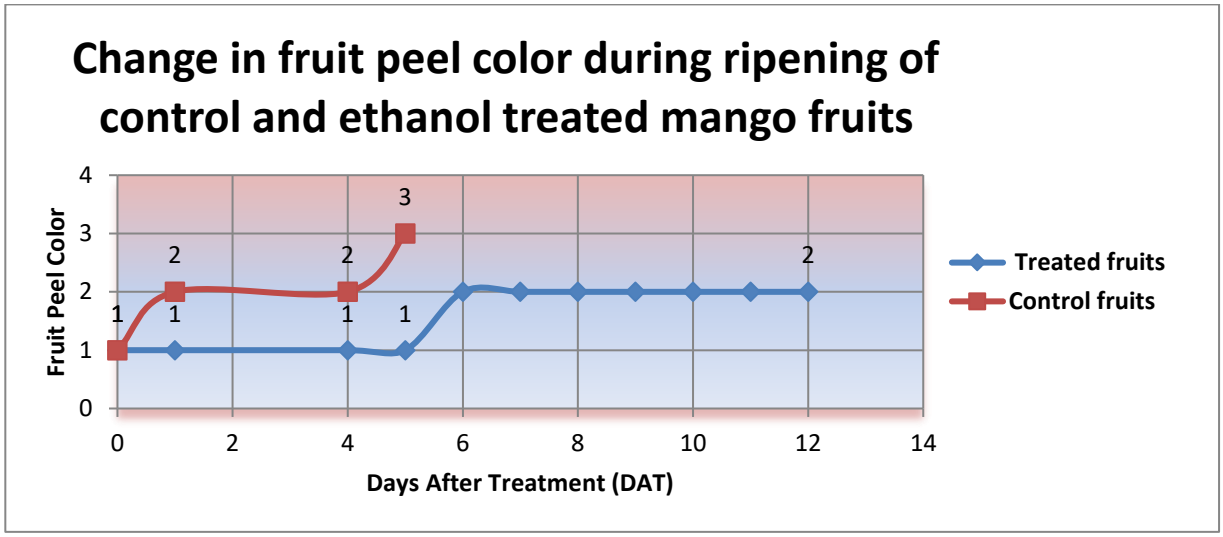


Figure 8: Change in fruit peel color during ripening of control and ethanol treated mango fruits

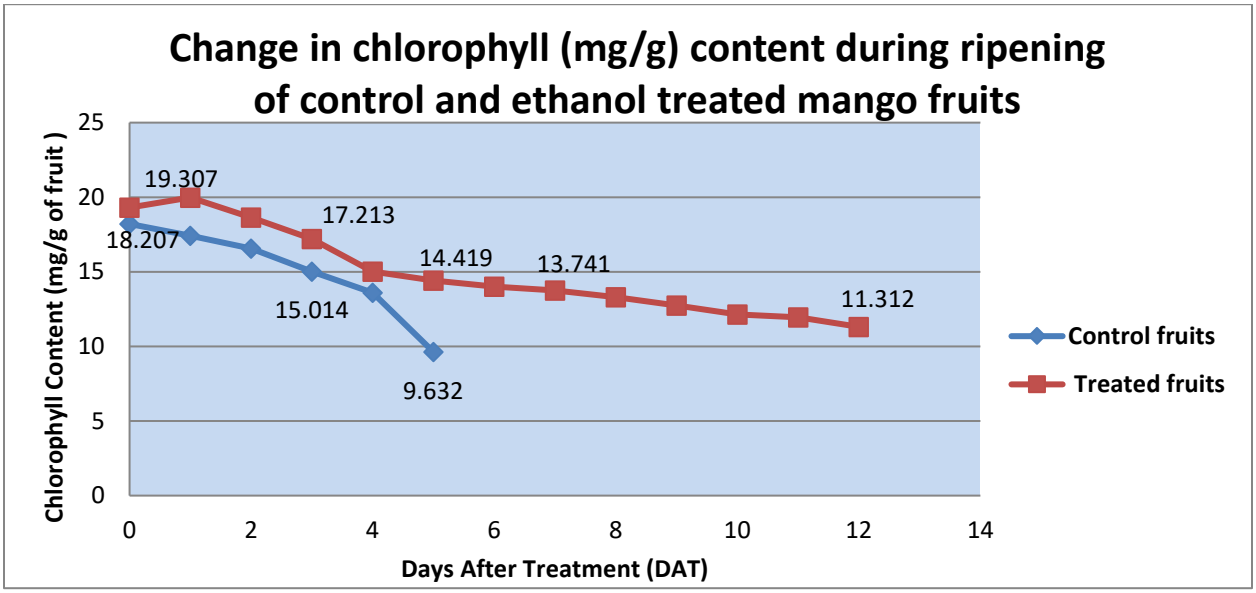




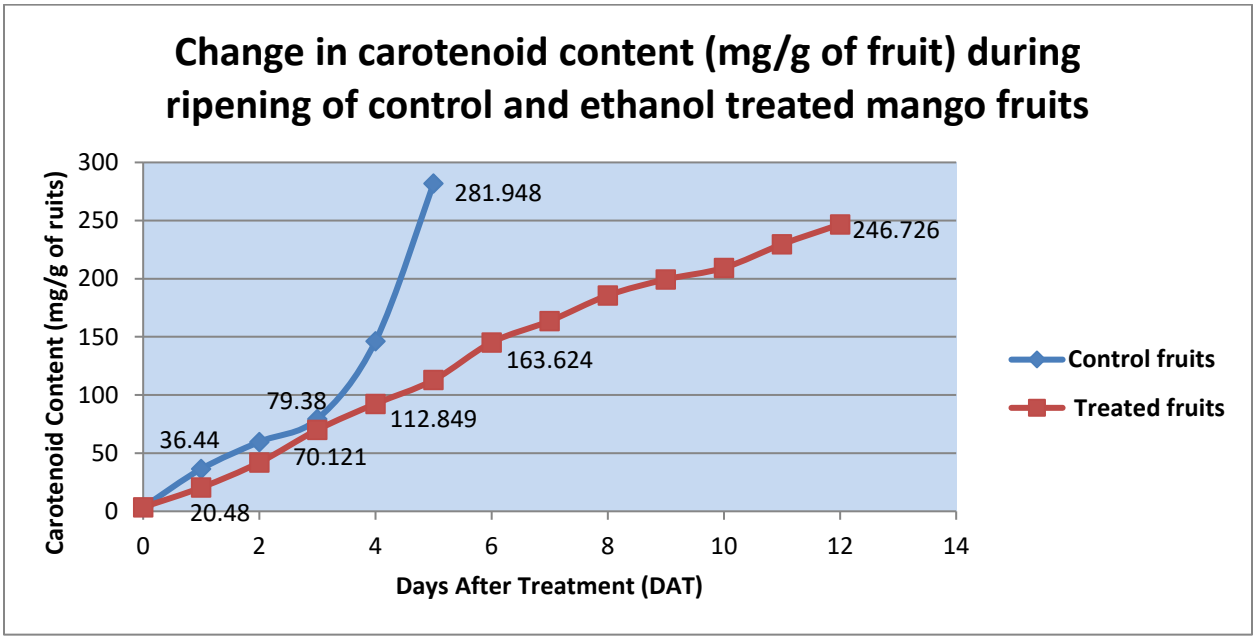
Graph 2: Change in fruit peel color during ripening of control and ethanol treated mango fruits

The green color of the unripe fruit is largely due to the presence of chlorophylls and the development of different colors during ripening is due to the disappearance of these chlorophyll pigments and biosynthesis of carotenoids. Control mangoes were able to retain green color rated 1 till 3rd day and on exhibited fruit peel color rated 3 on day 5 of achieving full ripeness. However, ethanol treated mango fruits were able to retain green color rated 2, even on the 12th day of their full ripe stage and were at stage 1 on 5th DAT (as shown in Graph 2).These results indicate that ethanol treatment delay the effect of chlorophyll breakdown and carotenoids biosynthesis.

3) Estimation of chlorophyll



Graph 3: Change in chlorophyll (mg/g) content during ripening of control and ethanol treated mango fruits



Graph 4: Change in carotenoid content (mg/g of fruit) during ripening of control and ethanol treated mango fruits.

Chlorophyll degradation and carotenoid biosynthesis are significant changes that take place during fruit ripening. There was significant decline in the chlorophyll content from unripe to ripe

stage of control mangoes (as shown in graph 3). On 5th DAT, total chlorophyll content of control fruits was observed to be 9.6 mg/g of fruit in comparison to ethanol treated fruits which had 1.5times higher chlorophyll content (14.6 mg/g of fruit). Even on the 12th DAT the chlorophyll content in ethanol treated fruits were higher (11.3 mg/g of fruit) than the control fruits on their full ripe stage on 5th DAT (9.6 mg/g of fruit).

On ripening chloroplast underwent extensive disorganization which was associated with the development of large osmiophilic globules [Knee,1972]. There was significant increase in the carotenoid content from unripe to ripe stage of control mangoes (as shown in graph 4). On 5th DAT, total carotenoid content of control fruits was observed to be 281.948 mg/g of fruit in comparison to ethanol treated fruits which had 2.4 times lesser carotenoid content (112.849 mg/g of fruit). Even on the 12th DAT the carotenoid content in ethanol treated fruit was lesser (246.76 mg/g of fruit) than the control fruits on their full ripe stage on 5th DAT (281.948 mg/g of fruit). A significant effect of ethanol in delaying carotenoid biosynthesis and chlorophyll degradation is observed.

4) Iodine test

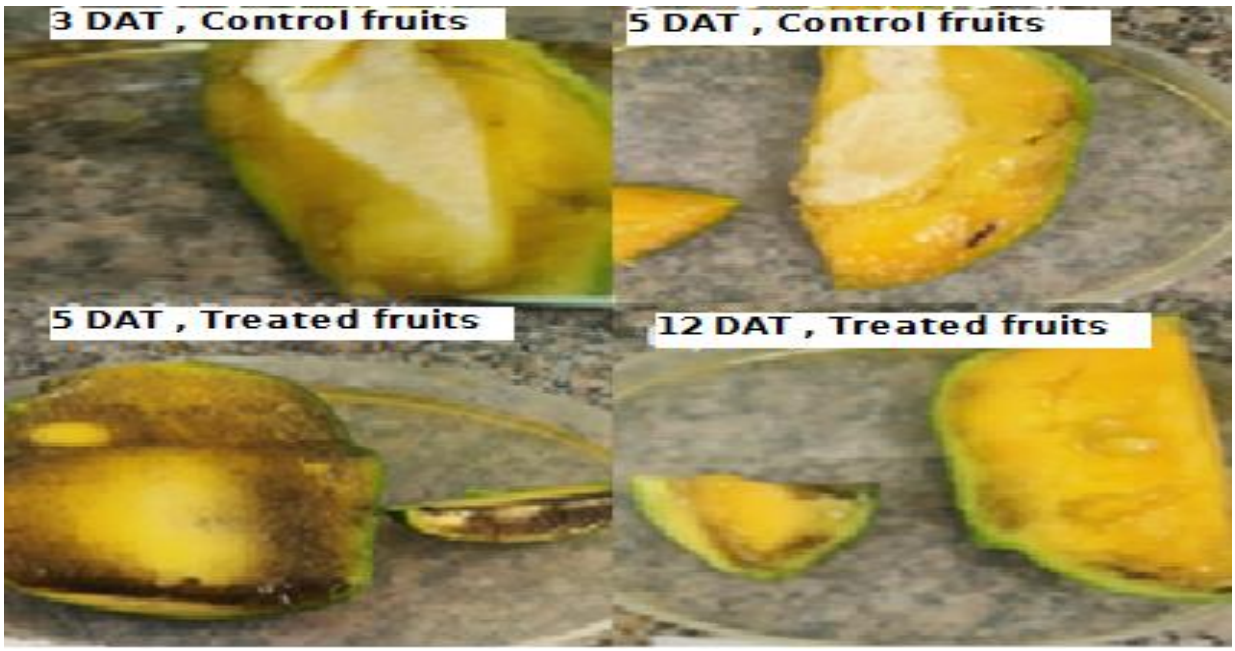


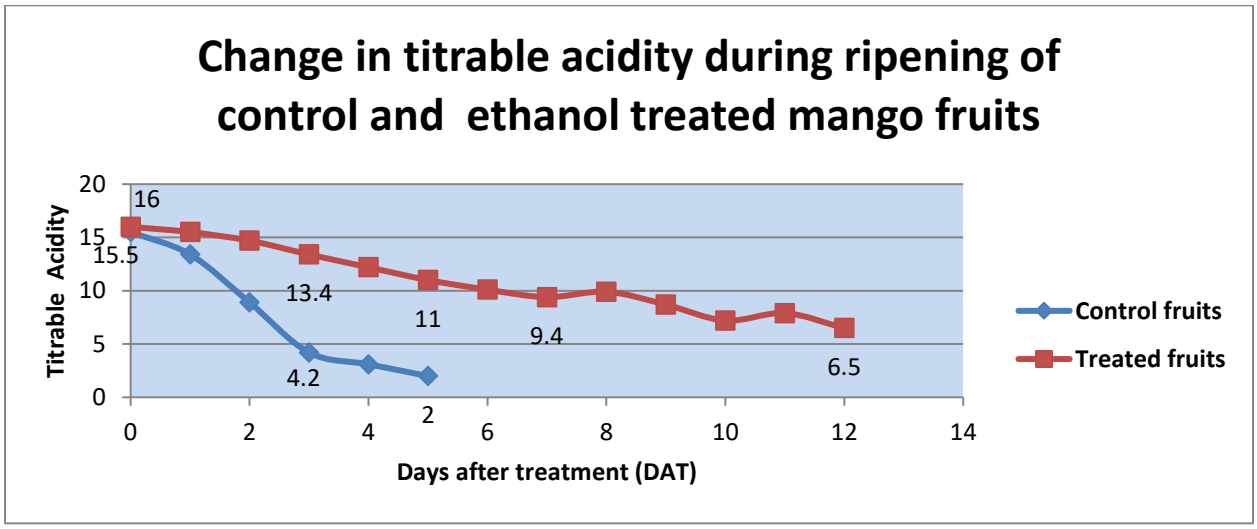
Figure 9: Iodine test for change in starch content during ripening of control and ethanol treated mango fruits

Starch is the main carbohydrate reservoir present in mature mango fruits. During mango ripening, starch is hydrolyzed by amylases to facilitate respiration [Matsumoto et al., 1983]. With ripening, starch disappearance can be studied using iodine test. Only traces of starch can be detected at the full ripe stage.

It is observed that the rate of starch disappearance was greater in the control fruits in comparison to ethanol treated fruits. On 5th DAT, there was complete disappearance of starch in control fruits, however ethanol treated fruits exhibited the presence of starch content after iodine test. Complete disappearance of starch content was observed only on 12th DAT in ethanol treated fruits (as shown in Figure 9).

Since the rate of disappearance of starch was much inhibited in ethanol treated fruits it may be concluded that ethanol delays the effect of amylases activity during fruit ripening.

5) Titrable acidity



Graph 5: Change in titrable acidity during ripening of control and treated mango fruits.

Titratable acidity content in the mango fruits show significant decline with ripening. Titrable acidity gives a measure of the amount of acid present. Citric acid along with other acids like tartaric acid is the major acid present in mango. The decline in acidity during ripening could be due to susceptibility of citric acid to oxidative destruction during respiration via Krebs cycle. The decline in acidity results in increase in pH which is confirmed with reduced titrable acidity. The observed decline in pH could be due to utilization of acids as respiration substrates. The decline in acidity during ripening was reported as a consequence of starch hydrolysis leading to increasing total sugars and acidity reduction [Anand et al.,2005].

It was observed that titrable acidity of ethanol treated fruits (13.4) was nearly3 times higher than control fruits (4.2) on 3rd DAT indicating faster rate of acid consumption during respiration in control fruits.Similarly, it was noted that titrable acidity of ethanol treated fruits (11) was 5.5 times higher than the control fruits(2) on 5th DAT.It is further observed that the acid content of fully ripe ethanol treated fruits(6.5) on 12th DAT was 3.2 times higher than acid content of fully ripe control fruits (2) on 5th DAT(As shown in Graph 5).

Since the titrable acidity in ethanol treated fruits are much higher than control it may be concluded that ethanol is having delaying effect on the respiratory breakdown of food reservoirs during fruit ripening.

CONCLUSION

Since acetaldehyde, and not ethanol, is the active component in inhibiting ripening, the conversion of ethanol to acetaldehyde by the tissue is the critical factor in determining whether a certain level of ethanol exposure inhibits ripening. For applied concentration of ethanol, delay in ripening is observed with an enhanced shelf life of treated fruits by 7 days. No change in natural aroma was observed due to ethanol treatment. Important conclusions drawn from the experiment are listed as:

1. Shelf life of ethanol treated mangoes was increased by 7 days in comparison to control fruits.

2. Ethanol treatment delayed the chlorophyll degradation by 7 days in comparison to control fruits.

3. Ethanol treatment reduced the respiratory loss in fruit weight by 7 days during ripening in comparison to control fruits. Fresh weight of ethanol treated fruits was noted to be 1.3times more than control fruits even after 7 days of ripening of control fruits.

4. Ethanol treatment inhibited the carotenoid biosynthetic pathway by 7 days.

5. Ethanol treatment reduced the respiratory consumption of acids by 3.25 times which is reflected in high titrable acidity of the treated fruits in comparison to fully ripened control fruits.

6. Ethanol treatment delayed the rate of starch consumption by 7 days in comparison to control fruits.

Since all the above ripening events are triggered by the hormone ethylene it may be concluded that ethanol is having delaying effect on ethylene biosynthetic pathway and its action. High dosage of ethanol treatment may be suggested for future experiments and for organoleptic

testing. The ability of ethanol to delay the ripening of climacteric fruits may to be dependent on a number of factors which probably include species, cultivar, maturity, applied concentration and mode of application.

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