TIU Transactions on Human Sciences (TTHS)

TTHS, Vol. 3, 2024

TIU Transactions on Human sciences

Role of Ethylene on the Ripening of Banana: The Effect of Ripening on the Soluble Sugar Content and Ferment Ability

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Abstract

Ripening of banana has a marked effect on the textural and physic-co chemical properties of fruits. With ripening there is conversion of starch to sugar and percentage of water extractable carbohydrate increases and that can be better utilized by yeast or fermentation. I case of climacteric fruits ethylene produced due to the metabolic action of fruit trigger the ripening process; it was observed that in controlled atmosphere storage with absorption of ethylene, shelf life of fruit increases with reduced fermentable sugar formation with long shelf life with respect to control. But due to wax coating of banana the rate of respiration decreases resulting less ethylene formation, but due to non penetrability of ethylene through the wax coating the adhered ethylene of surface causes more softening and faster generation of sugar.

Keywords: *Ethylene, Reducing sugar, Ferment ability, DNS, Ferrous Ammonium sulphate, S. cerevisiae*

1. Introduction

Ethylene significantly impacts several developmental events and plant responses to the environment. Biale and Young (1981)classified fruits as climacteric common-climacteric based on respiration patterns and the production of ethylene during maturation and ripening. It is widely acknowledged that ethylene is crucial to the ripening of climacteric fruits because is produced in significant amounts at the beginning of respiratory climacteric period and therefore ripening is induced by endogenous ethylene production and exogenous applied ethylene from the same production. Both 1-aminocyclopropane-1-carboxylic acid (ACC) synthase & ACC oxidase are activated in ripened climacteric fruits and play a pivotal role in regulating the production of ethylene [2, 3].The banana (scientifically known as *Musa spp.*), a typical climacteric fruit, produces more ethylene at the beginning during the climacteric period and then continues to do so while decreasing in accordance with alterations in respiratory functions, as the fruit reaches the full-ripe stage. However, unlike most of the climacteric fruits, banana fruit exhibits a sharp rise and fall in the rate of ethylene production during the early climacteric is of respiration [4,5,6]. For this reason , it is considered that the regulatory mechanism(s) of ethylene biosynthesis is in banana fruit may be different from that of other climacteric fruits. Therefore, it is important to investigate the possible mechanism(s) involved in the ethylene production and its characteristic effect in changing in the soluble sugar and its ferment ability in banana fruit samples.

1.1. Objective

To find the effect of ethylene on ripening of different types of bananas, especially ripe and unripe categories under different storage conditions, with sample-1 being untreated sample,sample-2 being controlled atmospheric type and sample-3 being wax coated modified one &their characteristic changes in the soluble sugar content and ferment ability of these banana samples

1.2. Ethylene Production and its Response in Climectaric fruits

There are basically two categories of ethylene biosynthesis: system1 refers to limited ethylene production during the pre-climacteric stage of climacteric fruit, and system 2 is exclusive to climacteric fruit and involves tremendous ethylene production that is auto-stimulated and known as "autocatalytic synthesis." Consequently, the presence or lack of autocatalytic ethylene synthesis is the primary ethylene-related distinction between climacteric and non- climacteric fruit [10, 11]. 1-aminocyclopropane-1-carboxylic acid (ACC) and S-adenosyl-L-methionine (SAM), are the ripening hormone produced from methionine. The biosynthetic route involve so many enzymes: ACC oxidase (ACO) which changes ACC into ethylene and ACC syntheses (ACS) which converts SAM into ACC. The corresponding genes have been identified and characterized [12, 13, 14]. Both ACO and ACS are encoded by a multigene family with expressions differentially regulated during fruit development and ripening [15].

While there is lesser evidence of genes regulating ethylene in banana, but in tomato there are studies that suggests LeACO1 and LeACO4 genes are up-regulated at the onset of ripening, and continue being active throughout ripening, LeACO3 displays only transient activation at the breaker stage of fruit ripening. It was demonstrated that Le ACS6 and LeACS1A are expressed during the pre-climacteric stage (system1), whereas LeACS4 and LeACS1A are the most active genes during the transition to ripening. Most active genes during the transition to ripening. LeACS4 then keeps up its high expression during the climacteric phase whereas LeACS1A's expression decreases. LeACS2 expression is induced and LeACS6 and LeACS1A expression are inhibited by the increase in ripening- associated ethylene production. It is believed that the transition from pre-climacteric system1 to climacteric system 2 depend on this fine-tuning of the ACS genes. It is noteworthy that autocatalytic production characterizes the shift to system 2, while ethylene's inhibitory feedback in its own biosynthesis route characterizes system 1. The down-regulation of ACO and ACS genes in transgenic plants through the use of an antisense method has proved unequivocally that ethylene is necessary to initiate the ripening of climacteric fruit. Strongly delayed ripening was seen in tomato [16, 17] and other fruits, such as melon [18] and apple [19], in the ethylene-suppressed lines. Even so, climacteric fruit has ethylene-independent ripening routes, as demonstrated by melon fruit, where ethylene suppression results in some softening, sugar buildup, and flesh pigmentation [20]. Based on these findings, it has been determined that in climacteric fruit, non-climacteric (ethylene-independent) and climacteric (ethylene-dependent) regulation coexist [21].

Differential screening techniques were used to identify and characterize ethylene-regulated genes due to the drastic alterations in the expression levels of several genes during the ripening of fruit and to obtain a better understanding of the control mechanisms governing this process [22]. Among the first genes sensitive to ethylene that were identified from tomato fruit were those that encoded enzymes involved in cell wall degradation, ethylene production, and pigment biosynthesis. Later, from mature green tomatoes that are susceptible to exogenous ethylene but do not yet produce increased levels of ripening-associated ethylene, a group of early ethylene-regulated genes was identified [23]. The concept that ethylene can function as a positive or negative regulator of gene expression is supported by expression studies that show the ethylene-responsive genes may appear temporarily activated, down-regulated, or up- regulated after brief hormone administration [24, 25]. Significantly, a large number of the earliest genes responding to ethylene encode hypothetical regulatory proteins implicated in transcriptional or as post-transcriptional regulation, transduction pathways, and other aspects of transcriptional control. This suggests that ethylene regulates their ripening process through a multifaceted and

intricate mechanism. The significance of ethylene regulation during tomato maturation was recently shown by a study [26]. The exact mechanism by which ethylene triggers the activation of every biochemical process linked to ripening is still unknown.

In the below figure (Fig.1), a focus is made on ethylene and auxin, aiming at exemplifying the importance of cross-talk between hormone signaling. Ethylene transduction cascade leads to the activation of EIN3-Like (EIL) genes, which activates primary target genes (ethylene-response factors, ERFs). ERFs in turn activate the expression of secondary ripening-related genes. Other signals, such as auxin, are also involved in this process. Some auxin response factors (ARFs) and Auxin/ indole acetic acid (IAA) transcription factors are also ethylene-

Responsive, and therefore are likely to participate in the expression of ripening related genes [27, 28].

2. Materials and Methods

2.1. Estimation of reducing sugar by di-nitro-salicylic acid (DNSA) method for unripe banana samples:

2.1.1. Principle

3, 5-Dinitrosalicylic acid (DNSA) is used extensively in biochemistry for the estimation of reducing sugars. It detects the presence of free carbonyl group (C=O) of reducing sugars. This involves the oxidation of the aldehyde functional group (in glucose)and the ketone functional group (in fructose). During this reaction DNSA is reduced to 3-amino5-nitrosalicylic acid (ANSA)which under alkaline conditions is converted to a reddish-brown colored complex which has an absorbance maximum of 540 nm.

2.1.2. Materials

Dinitrosalicylic acid reagent (DNS reagent):1gmof dinitrosalicylic acid, 200mg crystalline phenol and 50 mg sodium sulphite was added in 100 ml 1% NaOH solution. It was mixed using a mechanical shaker for 1 to 2 hours until it becomes a homogenized solution and then stored at 4 ℃ for further use.

2.1.3. Procedure

- 100 mg of the unripe banana samples under 3 different conditions, were weighed and the sugar was extracted with 80% hot ethanol twice (5 mL each time).
- The supernatant was collected and evaporated it by keeping it on a water bath at 80℃.
- 10 mL of distilled water was added to it for dissolving the sugars.
- ***For standards:*** 0.1 to 1 ml of the stock solution of 1mM dextrose solution were taken in test tubes with distilled water added to make the volume to 1 mL in each case, except the last one with1 mL stock solution. The blank contains 1 mL distilled water only. To all these, 1 mL of DNS reagent was added and then boiled for 10 min, and cooled and then added 8 mL of distilled water to make the volume to 10 mL. Finally, the absorbance was recorded at 540 nm.
- ***For samples:*** 1 mL of extract + 1 mL of distilled water + 1 mL of DNS reagent was taken, boiled for10min,cooled and 8mL of distilled water was added to them and the absorbance was recorded for each sample with at least 3 sets of random sampling data.

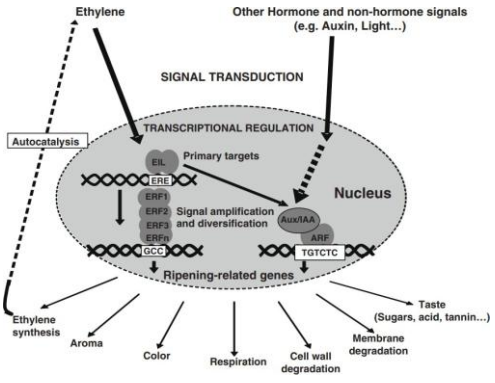

Fig. 1: Transcriptional regulation

2.2. Experimental Methodology for Fermentation of Ripe Banana (Edible part):

2.2.1. Sample preparation

Edible part of banana was mixer grinded with same amount of hot water and filtered using double layer muslin cloth to get the clear banana juice. This was used as the fermentation media in the below step.

2.2.2. Growth Media Preparation for yeast Saccharomyces cerevisiae:

100 mL of growth media was prepared with specification:

- Yeast Extract - 0.005%
- Peptone - 0.005%
- Glucose - 0.01%
- Water - 100mL
- pH – 5.0

Under ascetic condition, a test tube with 10 mL Luke warm distilled water with some dextrose was used to activate dry yeast and then adding it to the growth media. This was incubated at 29℃ for 24 hours under shaking condition with 200 rpm.

Fig. 2: *The untreated, controlled and modified samples with 3 sets of random sampling for each i.e., a, b and c for 3 different categories.*

2.2.3. Fermentation Media Preparation

200 mL of Banana juice was used in place of sucrose with specification:

- KH_2PO_4–0.1%
- $(NH_4)_2SO_4.7H_2O$–0.05%
- Yeast extract-0.01%
- pH- 4.5

To this fermentation media, 12 % of 200 mL = 24 mL growth media was added to it under ascetic condition using laminar hood. The conical was placed in incubation for 1.5 hours under shaking condition for aeration at 200 rpm and then under anaerobic condition for alcohol production for 30 hours.

Fig. 3: *Fermentation of untreated (sample 1), controlled (sample 2), modified (sample 3) for ripe banana storage trails*

Fig. 4: *Fermentation of samples*

2.2.4. To perform the back titration:

- Quantitatively the content so the Schott bottle was transferred to a 250 mL conical flask (using distilled water rinses).
- The burette was rinsed and filled with ferrous ammonium sulphate solution.
- The blank titration was performed using the prepared blank. It was titrated with ferrous ammonium sulphate until the solution turns to an emerald green.

- Then 5 drops of indicator solution was added to it.
- The titration was continued until the color changes from blue-green to brown. The endpoint is sharp and distinct.
- The blank titer was recorded.
- The sample titration was performed as the same way for the blank titration.
- The sample titer was recorded for 3 different samples [18].

Fig.7: Before titration

Fig.8:During titration

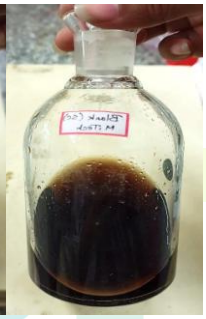

Fig.9:Endpointoftitration

2.2.5. Calculation of alcohol content using the following formula[18]:

Ethanol (%vol/vol) = 25-25[V_A/V_B]

Since, the above DNSA standard graph is having a R^2 value of 0.9809, so it can be considered as the standard for the sample values that are plotted on the same graph for sugar estimation of unripe banana samples with untreated sample having 0.1503 milli-mole concentration, controlled having 0.1183 mM concentration and modified having 0.2253 mM concentration. The standard graph was plotted using 1mM of dextrose concentration as working stock. As per the values, the controlled sample is having lesser sugar concentration as compared to other two, which means that the controlled sample has better shelf life and was not ripening as fast as other two samples.

3. Result & Discussion

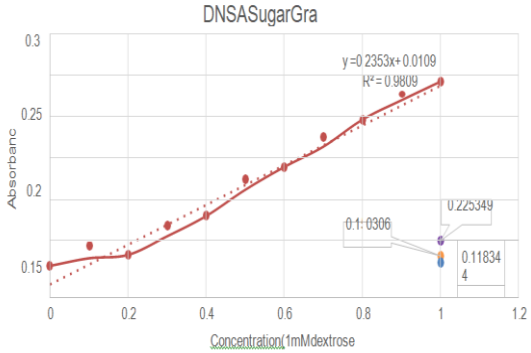

Fig.10:Dinitrosalicylic acid method standard graph with plotted sample values for estimation of reducing sugar in unripe banana trails of untreated, controlled, and modified samples.

Since, the above DNSA standard graph is having a R^2 value of 0.9809, so it can be considered as the standard for the sample values that are plotted on the same graph for sugar estimation of unripe banana samples with untreated sample having 0.1503 milli-mole concentration, controlled having 0.1183 mM concentration and modified having 0.2253 mM concentration. The standard graph was plotted using 1mM of dextrose concentration as working stock. As per the values, the controlled sample is having lesser sugar concentration as compared to other two, which means that the controlled sample has better shelf life and was not ripening as fast as other two samples.

Observation table for estimation of fermentable sugar in banana samples.

SampleName	Initial(mL)	Final (mL)
Dextrose standard(2mg/mL)	0	2
Untreated ripe banana sample(Sample-1)	0	1.7
Controlled atmosphere(Sample-2)withethylene absorber	0	2.8
Wax coated or modified banana(Sample-3)	0	3.9

Observation table for estimation of ethanol content in banana samples.

Sample Name	TiterValue(mL)	
	Day 2	Day3(Max ethanol production)
Untreated ripebananasample(Sample-1)	25.6	4
Controlled atmosphere(Sample-2)with ethylene absorber	20.5	1.5
Waxcoatedormodifiedbanana(Sample-3)	23.6	8.9
Blank	47.8	

4. Calculation

4.1. For Sample-1(Untreated ripe banana):

4.1.1. Soluble sugar estimation:

1 mL dextrose solution contain = 2 mg of dextrose

Or, 2 mL dextrose solution contain = 4 mg of dextrose

Or, 1 mL Fehling's solution contain = 4 mg of dextrose

Or, 1.7 mL of sample contain = 4 mg of dextrose

Or, 200 mL of fermented sample contain = {(200x 4)/ 1.7} =470.58 mg of dextrose

4.1.2. Total Ethanol content

Ethanol (% vol/vol) =25– 25[V_A/V_B]

Or, Ethanol (Day-2)=25–25(25.6/47.8)=11.61%

Ethanol (Day-3) = 25 – 25(4/47.8) = 22.907%.

4.2. For Sample-2 (Controlled atmospheric banana sample with ethylene absorber):

4.2.1. Soluble sugar estimation

1 mL of Fehling's solution contain = 4 mg of dextrose

Or, 2.8 mL of sample contain = 4 mg of dextrose

Or, 200 mL of fermented sample contain = {(200x 4)/ 2.8} =285.71 mg of dextrose.

4.2.2. Total ethanol Content

Ethanol (% vol/vol)=25– 25[V_A/V_B]

Or, Ethanol (Day-2)=25–25(20.5/47.8)=14.278%.

Or, Ethanol (Day-3)=25–25(1.5/47.8) = 24.215%.

4.3. For Sample-3 (Wax coated or modified banana sample):

4.3.1. Sugar solution estimation

1 mL of Fehling's solution contain = 4 mg of dextrose

Or, 3.9 mL of sample contain = 4 mg of dextrose

Or, 200 mL of fermented sample contain = {(200x 4)/ 3.9} =205.12 mg of dextrose.

4.3.2. Total Ethanol content

Ethanol (% vol/vol) =25– 25[V_A/V_B]

Or, Ethanol (Day-2)=25–25(23.6/47.8)=12.659%.

Ethanol (Day-3) = 25 – 25(8.9/47.8) = 20.345%.

5. Conclusion

In a nutshell, it can be concluded that ethylene can be triggered by many genes, and has autocatalytic activity which makes the effect of ripening even faster in climacteric fruits, especially in bananas. Ethylene regulation does affect the soluble sugar content of the fruits as it is responsible for many physico-chemical changes that occur during the onset of ripening in the post-harvest storage conditions. Moreover, the values from the experimental data for controlled atmospheric ripe banana samples seem to have better alcohol concentration characteristic value as compared to untreated and wax coated banana samples and also the sugar concentration in unripe banana sample was low, depicting that the ripening was slowed, thus increasing the shelf life by decreased ethylene absorption by the banana itself, in the controlled atmospheric storage condition.

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