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Harnessing Untapped Bio-Ethylene Source from Tomatoes Climacteric Effluent

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International Conference on Agricultural, Environmental and Biological Sciences (AEBS-2014) April 24-25, 2014 Phuket (Thailand)

SECTION-I

Harnessing Untapped Bio-Ethylene Source from Tomatoes Climacteric Effluent

Muhammad Makky, Konstantinos A. Paschalidis, and Peeyush Soni

***Abstract*—**A new method to tap the ethylene from fruit storage was proposed. Tomato fruits (Lycopersicum esculentum) c.v. Roma VF of different ripeness; breaker stage and red ripe, were put separately into four enclosed containers to the brim, and then stored in 20°C and 5°C. The proposed system connected effluent duct on each container to air compressor, syphoned gases from container into gas cylinder storage. The air duct on the container enables fresh air to flow in, substituting the suctioned gases. All air compressors were set to turn on for 1 minutes every two hours interval. The gas inside the cylinder was measured weekly using Gas Chromatography (GC) until the third week to analyze its main compound. The results showed that storing red ripe tomatoes in 20°C for 14 days produced highest ratio of ethylene to contaminants, thus provide the best combination of the system for tapping the bio-ethylene from fruits' climacteric effluent.

***Keywords*—**Tomato, Ethylene, Climacteric effluent, Gas Chromatography, Cold storage.

I. Introduction

ETHYLENE (C2H4) gas had been widely used for industrial purpose, such as polymerization, oxidation, halogenation, hydro-halogenation, alkylation, hydration, oligomerization, and hydroformylation [1]. It is generally ubiquitous in the environment, mainly arising from natural and sources, such as emissions from vegetation or climacteric fruits [2]. Today, most of bio-ethylene was produced from feedstock and biomass; nevertheless, new Bio-ethylene source produced from climacteric fruits, such as tomato, may become a promising alternative energy source in the future.

Tomato (*Lycopersicum esculentum*) is an edible fruits that had been consumed as one of many typical main diets throughout the world [3]. The fruits have rich lycopene content, that considerably beneficial to consumer health effects [4]. As a climacteric fruits, upon ripening, the fruits generate rapid increase in respiration, although the intensity and duration will be varied according to fruits' cultivar, storage temperature and atmospheric condition [5]. The presences of ethylene, as small as 0.1 ppm, will sufficiently enough to promote ripening in climacteric fruits [6], and when the fruits reached breaker stage, it started to produce sufficient ethylene [7].

Extending the self-life of tomato fruits through various techniques had been introduced in minimizing postharvest decay, by means of cold, heat, irradiation, or chemical applications, as well as Controlled Atmospheric (CA) conditions [8]. While heat, irradiation, and chemical applications may lead to alteration in fruits' aroma volatile profiles [9], CA storage treatments lead to reduction of volatile emission [10], decreased enzymatic activity [11], and increase fruits acidity [12]. On the other hand, storing tomatoes in lower temperature, especially in 5 and 20°C provide more practical solution while only slightly down regulated fruits climacteric process [13], [14]. These different temperature storage treatments were closely correlated to climacteric rates and fruits ethylene effluents [15]. Typically, tomato fruit produce ethylene in moderate amounts between one and ten µL kg-1 h-1 when stored in 20 °C temperature [16]. However, lower storage temperature (i.e. 5°C) subsequently reduced climacteric rate as well as ethylene production [17]. The fruits climacteric process indicated the final physiological progress, completion of maturation and of senescence process intervene [18]. This can be distinguished by the accumulation of pigment in fruits skin and softening of the fleshly parts in the fruits. With the completion of climacteric process, the fruits respiration rates will be decreased, and fruits produced lower CO2. In the same time, senescence process will lead to disintegration of fruits cell and rotting, and subsequently started to produce rotten gas (H2S) [19].

In this study, a new method was proposed to harness and stored bio-ethylene, generated by climacteric process of tomato fruits in the storage container. Different fruits ripeness stages (breaker-stage, and red-ripe), storage temperature (5, and 20°C), as well as storage time (7, 14, and 21 days) were tested, to determine the optimum climacteric process of tomatoes fruits under consideration. The experiment was set as random factorial with three replications. The best set up will be selected based on the highest bio-ethylene concentration obtained.

This study will provide a new breakthrough in the development of renewable energy alternative, and may be

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utilized as a pioneer of new bio-ethylene source.

II. Materials and Methods

A. Samples

Breaker-stage and red-ripe tomato fruits of c.v. Roma VF with uniform shape, size, and mass, growth under normal field conditions were harvested during the early period of 2013 winter in Thessaloniki area, Greece. The fruits were inspected carefully for defects and symptoms of disease, and when observed, were replaced immediately with healthy fruits. Only the best appearance fruits were selected in this study. Each fruits was weighed and identified.

B. Experiment Setup

The breaker stage fruits were placed into two transparent enclosed containers, each accommodate equal number of fruits. Limited headspace for each container was provided to allow gas circulation. The container then sealed, creating air-tight condition. For the proposed system, two holes, each 6mm in diameter, were drilled, the side walls, opposite to each other. The first hole was located in the upper part of the container wall, and the other was located in lower part, on the opposite wall. The first hole was functioned as intake air duck; enable fresh air entering into the container. The second hole was connected to mini air compressor (ARB, Australia), which siphoned air inside the container into standard gas cylinder. The first container was stored in 5°C, and the second was stored in 20°C. The compressor is set to automatically turn on for one minute every hour. For the red ripe fruits, the same arrangement was used.

C. Gas Chromatogram

The gas inside each gas cylinder was sampled every week, from zero until third week. For the analyses, One ml of stored gas was taken from the every gas cylinder, and then assessed using gas chromatogram (GC) (Agilent 7890B, USA), to identified the concentration of compounds under consideration inside the cylinder. The GC machine was equipped with a flame ionization detector (FID) and HP-PLOT Q Column model 19095P-Q04E (530 µm x 40 µm x 30 m). The inlet temperature was set to 60°C and the inlet pressure maintained at 6.8145 psi. The GC front detector FID was heated to 250 °C, and the GC Oven temperature was initially set to 60 °C, maintained for 2 min, then heated (30 °C min-1) until it reached 240 °C. The temperature in maintained for 1 min, and the total GC run time was approximately 8 minutes. A standard was used to calibrate the machine for measuring C2H4, CO, CO2, and H2S.

D. Statistical Analysis

A simple statistical analysis was used to identify significant difference between treatments, and to determine the best experimental set up combination for maximizing the climacteric process and C2H4 production while maintaining fruits shelf life.

The proposed experimental set up is described in the Fig. 1.

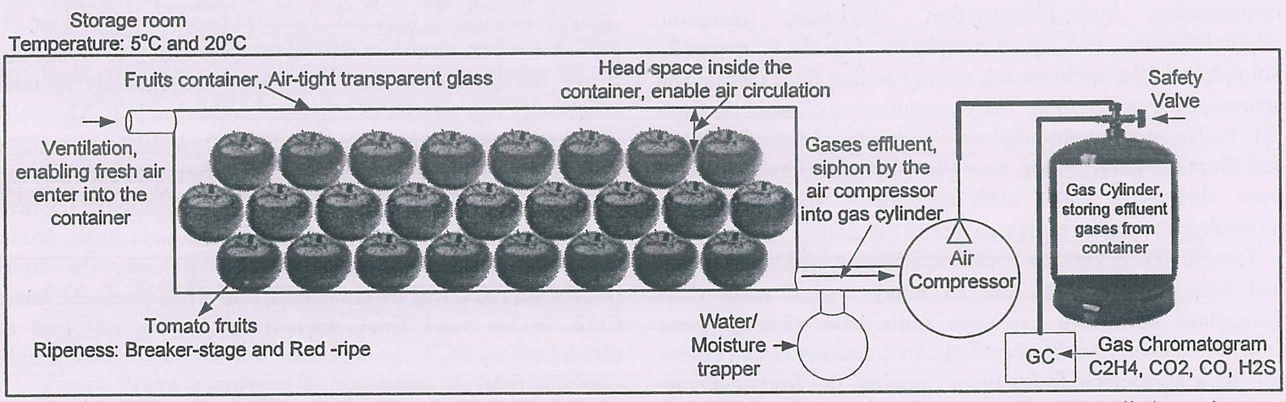


Fig. 1 The proposed system consists of cold storage room, fruits containers, fruits samples, air compressor, gas storage cylinder, and gas chromatography machine. Fruits were placed into containers, the effluent gases from container were siphoned into gas cylinder. Stored room temperature was set to 5°C and 20°C. Collected gas inside the cylinder was measured at 0, 7, 14, and 21 days using gas chromatography.

III. Results and Discussion

Fruits respiration upon storage was closely govern by temperature, atmospheric composition, and physical stress inside the storage room [20]. During fruits respiration, storage temperature regulated catabolism process, breaking down complex molecules into simpler molecules. In this process, fruits will uptakes oxygen from surrounding atmosphere and subsequently released energy, water vapor, ethylene, carbon dioxide, and many others simpler molecules to the environment. Temperature treatments for fruits storage had been widely developed in food processing industry, although it reserves prophylactic effects against physiological disorders [21]. The main risks encountered when storing fruit at low temperatures is chilling injury (CI). With appropriate management, CI can be prevented and fruits can be stored in lower temperature to allow significant longer shelf-life. This may promote lower transportation costs, such as by shipping, compared to current high-costs air-freight practiced. In addition, lower temperature storage had other advantages, such as disinfestations, maintaining fruits appearance and protection against other abiotic stresses [22].

Another factor that governs respiration is oxygen availability in surrounding atmosphere. Fresh air supply sufficient oxygen to support fruits respiration, however, in closed chamber, where air supply may restrict, and depletion of oxygen in the air reach minimum level (i.e. 2% or less), the fermentation will replace any respiratory process in fruits [23]. Subsequently, the starch and sugar in fruits were break down into sugar and furthermore, be fermented into alcohol and carbon dioxide, distinguishable by unpleasant flavor and premature senescent. Carbon dioxide level in head space gas inside the fruits storage room also plays important role, especially when the level rise above 5 percent [23], causing abnormal physiological conditions, such as non-ripening.

Most climacteric fruits produce ethylene auto-catalytically [24], especially during climacteric ripening, although the concentration may vary significantly between fruit. Higher ethylene concentration in fruits will promote greater respiration rate and metabolic activity, and accelerate biochemical and physiological transformations during ripening. Genetic factor, harvesting time and conditions, will pre-determine fruits' ethylene production, in time and amount, as well as its climacteric respiratory [20].

The respiration rate in tomato also closely related to its weight and ripeness stages [25]. The highest respiration was observed in immature fruits, then declining along the ripening development after climacteric respiratory take place. Thus respiration can be used to forecast fruits metabolic activity, its ripening stages and shelf life [26]. By lowering respiration rate through temperature treatment, the potential shelf life of the fruit can be extended. For tomatoes, where the ripening process is accompanied by the burst of respiration and ethylene production, lowering its storage temperature will subsequently uphold its climacteric processes, delayed the physiological transition from maturity to senescence, thus increasing its self-life.

Initially, the ethylene production of fruits will be low within the first 24 hour [25] after harvest. Over the time course, the production will significantly increase, and will be at the peak within seven days of harvest, corresponding to climacteric activity. Therefore, the best practiced was to keep the fruits directly in low temperature storage after harvest. Delayed in proper handling will reduce fruits shelf life exponentially, and degrade quality upon consumption.

Ethylene measurement can be performed by gas chromatogram sampling with a syringe. However, individual fruit sampling may not be the best practiced, considering time and resource required. Instead of accurate measurement, the main problem faced when sampling fruit ethylene is the unfathomable sample number to be assessed [25]. In this study, the ethylene is measured by sampling the head spaces gas inside the fruits container by means of chromatograms. Since the fruits have uniform shape, size, and maturity, this method can appropriately be considered as accurate approach to depict each fruits physiological state and indicate their proper regime upon storage. Sampling results from the head space gas provided comprehensive data of ethylene gas (C2H4), carbon dioxide (CO2), carbon monoxide (CO), and hydrogen sulfide (H2S) produced by samples along the storage. Standard gases with known concentration were used to quantify the results. Total gas concentration was divided by the quantity of the samples in order to enable data to be considered as individual measurement. Although the GC machine could provide precision results, nevertheless, there always exists possibility that several individual fruit could be riper than the rest of the bulk, generating more ethylene than the others [25]. The results of gas chromatogram sampling in this study was given in the Table. I

TABLE I
SAMPLES CLIMACTERIC PROCESS EFFLUENTS IN THE GAS CYLINDER AS DETERMINED BY GAS CHROMATOGRAM

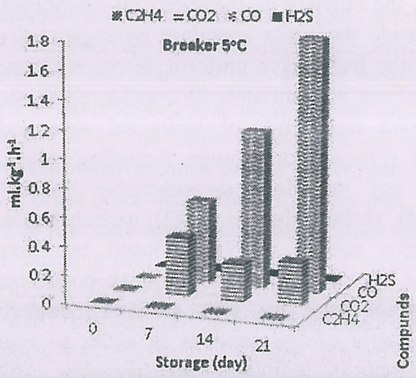
Ripeness Stage	Gas Concentration [z] ($ml.kg^{-1}.h^{-1}$)															
	Day 0 (1h)				Day 7				Day 14				Day 21			
	C2H4	CO2	H2S	CO	C2H4	CO2	H2S	CO	C2H4	CO2	H2S	CO	C2H4	CO2	H2S	CO
Breaker (5°C)	0.001a	0a	0a	0a	0.0068a	0.413a	0.017a	0.593a	0.006a	0.26a	0.01a	1.12b	0.005ab	0.3a	0.01a	1.79b
Breaker (20°C)	0.011ab	15.07b	0a	0a	0.015ab	12.55b	0.02a	3.73b	0.002a	0.91ab	0.011a	1.84b	0.0003a	1.05ab	0.009a	0.65a
Red (5°C)	0.011ab	3ab	0a	0a	0.02b	3.148ab	0.104b	3.52b	0.03b	6.65b	0.052b	0.66a	0.04b	10.57b	0.041b	0.334a
Red (20°C)	0.016b	15b	0a	0a	0.011ab	9.1b	0.1b	0.25a	0.013ab	0.76ab	0.052b	0.33a	0.002ab	0.54a	0.04b	0.57a

[z] Means followed by the same letter within columns are non-significant at $P = 0.05$ by Duncan's multiple range tests.

For the breaker stage fruits, different storage temperature determined the C2H4 production, respiration rate (CO2), and anaerobic process (CO). However, no significant different was observed in hydrogen sulfide produce by fruits. Storing the fruits in 20°C made C2H4 production higher in the first two observations, 0 and 7 days, compare to fruits stored in 5°C. This condition was reversed when the samples were measured on 14 and 21 days. Lowering storage temperature to 5°C in breaker stage fruits resulted in steady slow production of C2H4 from day 7 onward. Similar conditions also observed in fruits respiration. Fruits respiration rates in the first week were significantly higher when stored in warmer temperature (i.e. 20°C), however, after the 7th day, the fruits respiration were dropped and only slightly higher compared to lower storage temperature (i.e. 5°C). The rapid increase of CO was observed when fruits were stored at 20°C after 7 days, and then decreased afterward. On the other hand, the CO production in fruits stored at 5°C was steadily increased along the storage.

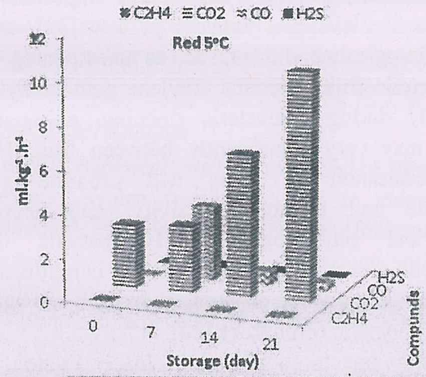
In red ripe fruits, storing the samples in lower temperature (i.e. 5°C) generated higher C2H4 and CO production, except in the initial time (C2H4 and CO), and on 21st day (CO). No significant differences were observed for H2S production in both treatments, suggesting different storage temperature has no effect to this gas output for the red ripe fruits. In additions, the respirations of the samples showed paradox behavior for different temperature treatments. Low temperature storage promotes increase of CO2 production, especially after the first week, while warmer storage temperature upholds the fruits respiration after the second week. This condition suggest that storing red ripe tomato in 20°C induced ripening acceleration and brought the fruits into senescence stage in shorter time, thus subsequently reduce the fruits shelf life.

The results indicated that the climacteric process in tomato fruits was strongly affected by its storage temperature and ripeness stages when harvested. All four parameter measured showed different behavior in each set of samples, according to its ripeness and storage temperature. These behaviors were described in Fig. 2. In most of the treatments, respiratory process was predominant, except for fruits stored at 5°C in breaker stage, where CO production showed the most prominent gaseous effluent.



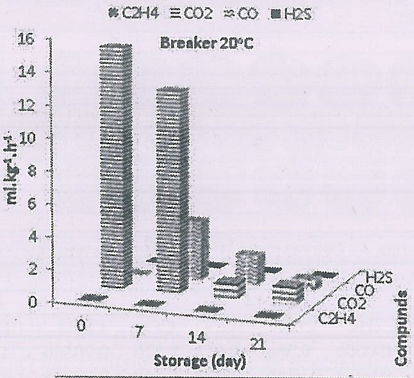
	0	7	14	21
C2H4	0.001	0.007	0.006	0.005
CO2	0.000	0.412	0.259	0.304
CO	0.000	0.593	1.116	1.792
H2S	0.000	0.017	0.011	0.009

(a)



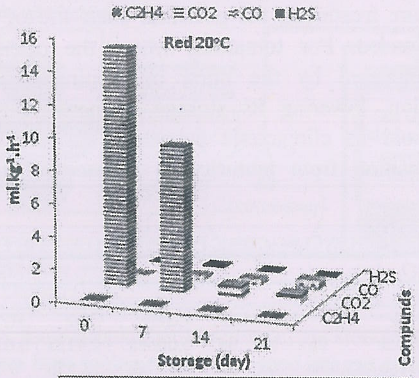
	0	7	14	21
C2H4	0.011	0.020	0.031	0.037
CO2	3.000	3.148	6.654	10.573
CO	0.000	3.529	0.660	0.334
H2S	0.000	0.104	0.052	0.041

(b)



	0	7	14	21
C2H4	0.011	0.015	0.001	0.000
CO2	15.067	12.546	0.911	1.046
CO	0.000	3.733	1.837	0.645
H2S	0.000	0.017	0.011	0.009

(c)



	0	7	14	21
C2H4	0.016	0.011	0.013	0.001
CO2	15.000	9.101	0.761	0.540
CO	0.000	0.248	0.327	0.572
H2S	0.000	0.104	0.052	0.041

(d)

Fig. 2 Climacteric behaviors of tomato fruits of different ripeness stored in different temperature showed the characteristic of ethylene, carbon dioxide, carbon monoxide, and hydrogen sulfide effluent rate accordingly for fruits stored at 5°C in (a) breaker stages and (b) red ripe stages, and for fruits stored at 20°C in (c) breaker stages and (d) red ripe stages.

To determine the best combination of storage conditions and fruits' ripeness stage for maximizing the ethylene production, all four parameter that had been measured need to be assessed properly. Not all the gas effluent from the storage fruits was desired to be high in concentration, because apart from ethylene, other gas produced by fruits in the storage can be considered as contaminant, and eventually, the ethylene obtained in the process must be purified to remove these contaminants before it can be used as industrial gas or renewable energy sources. Therefore, the best combination will be the storage conditions and fruits ripeness that enable maximum ethylene production while have minimum contaminants. From Fig. 3, the highest ethylene production obtained when red ripe tomato fruits were stored at 5°C for 21 days. However, at the same time its CO2 was among the highest of other storage combinations. On the other hand, the combination of red ripe fruits and 20°C storage temperature provide better ethylene to contaminant ratio if stored for 14 days (Table II.), and thus, will be the best arrangement to be set for harnessing the ethylene for industrial purpose or as renewable energy source

The ethylene gas obtained from the fruits climacteric process in this study will be required further purification. One of the techniques that can be applied is purification process by streaming ethylene into low temperature absorbents to filter impurity, mainly H2S gas. The gas will require further processing that involves hydrogenation process using high activity removal catalyst. The next step in ethylene purifying was removal of CO gas by injecting oxygen (O2) into the gas container. Both gas will work against each other and change their chemical compound into CO2, which followed by re-oxidizing process. Two reactors may be used to clean moisture from the ethylene gas. And finally special adsorbent will be used to clean the remaining CO2.

TABLE II
TOMATO FRUITS ETHYLENE TO CONTAMINATION PRODUCTION RATIO UPON STORAGE WITH DIFFERENT RIPENESS, TEMPERATURE, AND TIME

Ethylene to contaminants ratio (ml/l)				
	Storage Time (day) ^{z}			
Ripeness	0	7	14	21
Breaker 5°C	0.001a	6.606b	4.316ab	2.158b
Red 5°C	3.572b	2.975ab	4.236ab	3.401b
Breaker 20°C	0.737ab	0.927a	0.543a	0.175a
Red 20°C	1.054ab	1.190a	11.038b	1.283b

^{z} Means followed by the same letter within columns are non-significant at P = 0.05 by Duncan's multiple range tests.

This study will lead a new way of bio-ethylene exploitation by utilizing fruits' climacteric process, which until now, still remained as untouchable sources. With the increasing demand of ethylene from industry, so far, it was mainly being generated from petroleum based chemicals; this study will open new insights of possibility to tap bio-ethylene, which can be also be used as an environmentally friendly and renewable. energy source. In the long term, this could help reducing petroleum consumptions and dependence, and substituted petroleum-based products with the bio-ethylene based industry. In addition, by exploring the renewable ethylene source, industry could reduce their greenhouse gases emissions from burning fossil fuels.

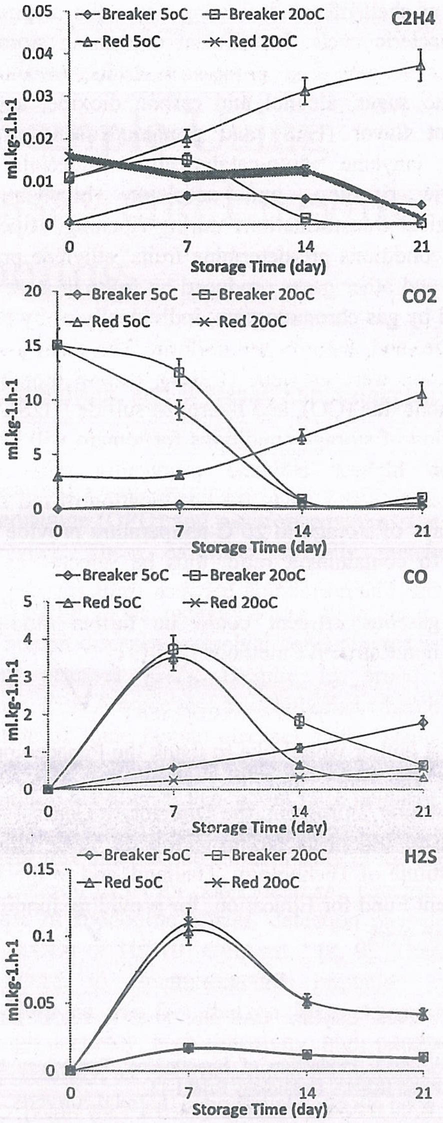


Fig. 3 Ethylene and other gaseous effluent from tomato fruits climacteric process according to chemical compound.

This Bio-ethylene gas, produced by tapping the effluent of fruits climacteric process, can be further purified in order to be reused as renewable energy sources, or as ripening agent for agriculture sector and post-harvest. With knowledge of the best combination to get the maximum bio-ethylene tomato fruit storage, this study will also open up the possibility for the development of equipment based on this method, obtaining higher ethylene concentration and increased efficiency and productivity.

IV. CONCLUSION

The respiration of climacteric fruits was regulated by storage temperature, atmospheric composition, and physical stress. Storage temperature influenced catabolism process in the cells, breaking down complex molecules into simpler molecules. Storing fruits in lower temperature allow significant increase of shelf-life, and slowing down the respiration rate and climacteric cycle. Insufficient oxygen in storage room commence fermentation process in fruits, breaking down starch into sugar, alcohol and carbon dioxide, and release unpleasant flavor (H2S) and premature senescent. Fruits produce ethylene auto-catalytically, especially during climacteric ripening, and accelerate biochemical and physiological transformations during ripening. Ripeness and physical conditions pre-determine fruits' ethylene production. Ethylene and other gases produced by fruits in store could be measured by gas chromatogram, individually or by bulk if the shape, size, and maturity are uniform. The main gases under consideration were ethylene (C2H4), carbon dioxide (CO2), carbon monoxide (CO), and hydrogen sulfide (H2S). The best combination of storage conditions for tomato will be selected based on highest ethylene production with minimum contaminants. In this study the combination of red ripe fruits and 14 days of storage in 20°C temperature provide optimum ethylene to contaminant ratio, thus be selected as the best arrangement. The correlation between fruits ripening progress and its gaseous effluent could be further studied using available nondestructive methods [27]-[31]

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