Identification and Measurement the Extent of the Chemicals Used for Early Ripening and Preservation of Frits in the Southwestern Region of Bangladesh

การจำแนกและวัดปริมาณสารเคมีที่ใช้ในการบ่มและแปรรูปผลไม้ในภูมิภาคตะวันตกเฉียงใต้ของประเทศบังคลาเทศ

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Abstract

An experiment was conducted at the Horticulture Laboratory in Khulna University, Khulna, Asia Arsenic Network Laboratory, Jessore and Pesticide Analytical Laboratory, Entomology Division, BARI, Gazipur, of Bangaldesh during the period from April to July, 2011. The objectives of the study to measure and identify the harmful chemicals (Calcium Carbide, Formalin, Ethefon) used for ripening and preservation of fruits in Jsssore, Khulna and Satkhira markets. In Jessore, Khulna, and Satkhira markets, calcium carbide was found 80%, 70% and 70% mango samples and 40%, 50% and 60% banana samples, respectively. From the same markets no carbide was found in papaya samples. Each of the Jessore and Satkhira markets no carbide was found in any jackfruits but from Khulnamarket 20% jackfruits contained calcium carbide. In Jessore markets, 20% mango, 5% banana and 2% papaya samples contained formalin. In Khulna markets 25% mango, 6% banana and 3% papaya samples contained formalin residue. It was found from Satkhira markets that 15% mango and 3% banana samples contained formalin. It was revealed that in Jessore, Khulna and Satkhira markets 80-100% mango, 40-50% banana and 30-40% papaya samples contained ethefon residue. From the above results it was revealed that maximum fruits were treated with chemicals. Therefore, it can be suggested that it is important to increase awareness in consumers and steps should be taken by the government or policy makers against the adulteration of fruits.

Keywords: Identification, Measurement, Calcium carbide, Formalin and Ethefon.

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บทคัดย่อ

งานวิจัยนี้ได้ทำการทดลองในห้องปฏิบัติการหลายแห่งด้วยกัน ได้แก่ ห้องปฏิบัติการพืชสวนของมหาวิทยาลัยขุลนา เมืองขุลนา เครือข่ายห้องปฏิบัติการสารหนูเอเชีย ห้องปฏิบัติการเจสซอร์ และห้องปฏิบัติการวิเคราะห์ยาฆ่าแมลง กองกีฏวิทยา BARI เมืองคาชีปุระ ประเทศบังคลาเทศ ในช่วงเดือนเมษายนถึงเดือนกรกฎาคม 2554 โดยมีวัตถุประสงค์เพื่อจำแนกและวัดปริมาณสารเคมีอันตราย (เช่น แคลเซียมคาร์ไบด์ ฟอร์มาลีน อีทีฟอน เป็นต้น) ที่ใช้ในการบ่มและแปรรูปผลไม้ในตลาดในเมืองเจสซอร์ ขุลนา และสาขิรา จากการตรวจตัวอย่างมะม่วงในตลาดเมืองเจสซอร์ ขุลนา และสาขิรา พบสารแคลเซียมคาร์ไบด์ร้อยละ 80 70 และ 70 ตามลำดับสำหรับการตรวจตัวอย่างกล้วยในสามเมืองดังกล่าวพบว่าร้อยละ 40 50 และ 60 ตามลำดับมีสารแคลเซียมคาร์ไบด์ปนเปื้อนโดยไม่พบสารคาร์ไบด์ในตัวอย่างมะละกอในตลาดทั้งสามเมือง อย่างไรก็ดี พบสารแคลเซียมคาร์ไบด์ปนเปื้อนร้อยละ 20 ในตัวอย่างขนุนในตลาดเมืองขุลนา แต่ไม่พบในตลาดเมืองเจสซอร์และสาขิรา ร้อยละ 20 5 และ 2 ของตัวอย่างมะม่วง กล้วย และมะละกอตามลำดับในตลาดเมืองเจสซอร์พบการปนเปื้อนของฟอร์มาลีน ซึ่งร้อยละ 25 6 และ 3 ตามลำดับของตัวอย่างผลไม้ทั้งสามชนิดดังกล่าวในตลาดเมืองขุลนามีฟอร์มาลีนตกค้าง สำหรับตัวอย่างมะม่วงร้อยละ 15 และตัวอย่างกล้วยร้อยละ 3 ของตลาดในเมืองสาขิราพบการปนเปื้อนของฟอร์มาลีน นอกจากนี้ ร้อยละ 80-100 ของตัวอย่างมะม่วง ร้อยละ 40-50 ของตัวอย่างกล้วย และร้อยละ 30-40 ของตัวอย่างมะละกอในตลาดเมืองเจสซอร์ ขุลนา และสาขิรา พบสารอีทีฟอนตกค้าง ผลการสำรวจข้างต้นแสดงให้เห็นว่ามีการใช้สารเคมีในผลไม้ส่วนใหญ่ดังนั้นการสร้างความตระหนักให้แก่ผู้บริโภคในการป้องกันอันตรายจากสารเคมีตกค้างจึงเป็นสิ่งสำคัญอย่างยิ่งรวมทั้งรัฐบาลหรือผู้กำหนดนโยบายควรมีมาตรการในการควบคุมการใช้สารเคมีในการบ่มผลไม้อย่างเหมาะสม

คำสำคัญ : การจำแนก การวัดปริมาณ สารแคลเซียมคาร์ไบด์ สารฟอร์มาลีน และสารอีทีฟอน

Introduction

Bangladesh is predominantly an agricultural country comprising 1, 47, 570 sq. km of area with an estimated population of more than 140 millions (BBS, 2012). Its population density is 993 per sq. kilometer (BBS, 2011). During ripening of fruits, the fruit businessmen unconsciously try to apply increased amount of ethylene (Saifuddin, 2012). But they do not maintain the proper dose of the chemical. Some dishonest retailers or businessmen use calcium carbide, formalin and ethylene. But the over doses of these chemicals are harmful for human body. The presence

of harmful chemicals in fruits always draws scientific concern as these are considered responsible for affecting health. The increasing trends of chemicals are largely attributed by the use of harmful chemicals for early ripening or preservation of fruits (Ghose, 1997). Calcium carbide (CaC2), formalin (37% H-CHO solution) and ethefon (C2H6ClO3P) are harmful chemicals for human body (Anonymous, 1992). Calcium Carbide causes stomach problem and long time uptake causes kidney and liver damage, sometimes skin or lungs problem and allergy and mutagenic affects on human body (Kanti and Begum, 2011). Carbide is also harmful for pregnant mothers. Formalin causes cancer, liver damage, kidney or lungs problem, allergy and mutagenic affects on human body. Mango, banana, papaya, and jackfruit samples were examined in laboratory to identify the residual effect of Calcium Carbide, Formalin and Ethefon.

Objective

To identify and measure the extent of chemicals used for early ripening and preservation of fruits by Atomic Absorption Spectrophotometer Method, Formalin tester method and GC-2010 FTD machine method.

Material and Methods

The present investigation was carried out in the Horticulture Laboratory in Khulna University, Khulna and Asia Arsenic Network Laboratory, Jessore, Bangladesh during the period from April to July, 2011. Some fruits (mango, banana, papaya, Jackfruit) were collected from the local markets (Jessore, Khulna and Sathkhira) and used the pulp of that fruits for analysis. Calcium carbide was measured by Atomic Absorption Spectrophotometer method and Formalin was identified by using formalin tester (invented by Faruk Bin Hossain, Marketed by Shwapno Bangla Agro Care, 2012) in the laboratory. Data recorded on different parameters were statistically analyzed for ANOVA using MSTAT-C computer software.

Calcium Carbide measurement

Calcium carbide or Nishadol when come near the atmospheric moisture then it produce acetylene gas. The reaction is, $CaC_2 + H_2O \longrightarrow Ca(OH)_2 + HC \equiv CH$, So if we measure the amount of calcium hydroxide from the fruit samples and

compare it's amount with fresh fruit sample/ideal pure fruit sample then we can say, whether calcium carbide was treated on that fruits or not.

Atomic Absorption Method (Calcium Carbide Measurement)

Another ten fruits of mangos, bananas, papayas and jackfruits were collected from the markets (Jessore, Khulna and Satkhira). The samples were analyzed followed the methods of (Anonymous, 2011).

Reference materials: i) Flame Atomic Absorption Spectrophotometer (Shimadzu Co. Germany, Model 6200) ii) Nitric acid iii) Sulfuric acid iv) Beaker v) Volumetric flask etc.

a. Nitric acid decomposition: At first 2-5g of fruit sample was grinded and air dried and then placed in a conical flask. It was wetted with water and added 25ml of nitric acid. Next, it was gently heated to start a sample reaction. After cooling (About 1 hour) 10 ml of sulfuric acid was added and gently heated to concentrate (Anonymous, 2011). Midway, if the contained material became dark, 2-3 ml portion of nitric acid was added and continued heating. When the contained material turned yellowish or colorless, decomposition was completed (needed about 2 hours). After cooling, 2 ml of hydrochloric acid was added, and used water to prepare fixed volume of measurement solution.

b. Preparation of standard solution: The samples were calibrated against four pointed calibration curve (0 ppm, 0.5 ppm, 1.0 ppm and 2.00 ppm) of standard solution of Ca.

Formalin examination: The materials used for the study were fresh fruits from the local markets and the 'Formalin tester' collected from Shwapno Bangla Agro Care, Co. Dhaka. Some fruits were treated by Formalin (37% formaldehyde) and examined by 'Formalin tester' and observed the colour change. The blank tests were done by using fresh fruits collected from known gardens, not from commercial market.

Sample Preparation

Each of the samples (fruits) were washed with about 4 ml of water and kept the water into a small test tube.

Step 1

About 2 ml of the washed water was taken into the test tube.

Step 2

One drop of chemical (Formalin tester solution) was added into the test tube and it was shaked for 20 seconds and kept it to observe the colour change.

Observations

(i) If the color of wash water of the sample changed into yellowish or colourless, it means presence of formalin.

(ii) If the colour of solution remains blue or greenish that means formalin is not present

Determination of ethefon

This study was carried out in the Pesticide Analytical Laboratory, Entomology Division, BARI, Gazipur. Ten fruits of mango, banana and papaya were collected from the market of Jessore, Khulna and Satkhira for analysis to determine Ethefon. Sampling was done during 30 April to 30 July, 2011. The samples were analyzed following the method of Rahman et al. (2011).

Materials

GC-2010 equipped with Flame Thermoionized Detector (FTD) was used for analysis of ethefon. The standard for the ethefon was obtained from Siga-Aldrich Laborchemikalien, Gbh P. O. Box-100262, D-30918, Seelze, Germany via Bangladesh Scientific Pvt. Ltd. Dhaka, Bangladesh. The standard contained >99.6% purity. The mango, banana and papaya samples were collected from the markets of Southwestern region of Bangladesh (Jessore, Khulna and Satkhira).

General Information about Ethefon

Common Name : Ethefon

Empirical Formula : C2H6ClO3P

Chemical Names : 2-chloroethylphosphonic acid (IUPAC)

Trade Name : Ethrel, Ripen, Rizen, Harvest, Promot, Riten, Etheplus, L-PEN 25, Cerone, Florel, Bromeflor, Flordimex, Ethephon, Ethefon, Terpal etc.

Sample Extraction

At first the pulps of mango, banana, and papaya samples were separated by a sharp knife and it was blended thoroughly with a blender (Handmixer M-122,

Bamiz, Switzerland). Then a sub sample of 25g from each fruit was taken into a wide mouth jar and 100ml of hexane was added. Then 1.0ml of prepared diazomethane (trimethylsilyl) solution was added for Methylation of ethefon. Sodium sulphate (Na2SO4) was also added (as required) in the sample until water was removed from the sample. The mixture was then macerated with high-speed homogenizer (Ultraturax, IKA T 18 basic, Germany) for 2 minutes. The homogenized material was then poured into 250ml conical flask and placed into shaker (Orbital Shaking Incubator, Rexmed, Sweden) for 12 hrs continuous shaking. After shaking, the slurry was filtered through a Buchner funnel with suction. The flask and filtered cakes were rinsed with 25ml of hexane. The filtrate was then transferred into 250ml round bottom flask and was dried to 3-5 ml by evaporation using a rotary vacuum evaporator (Laborota-4001, Heidolph, Germany). The concentrated filtrate was then transferred into volumetric flask making 10ml in volume. For colour removal, 20ml methanol was added with 10ml filtrate and shaked thoroughly for 3-5 minutes. After shaking, the separator funnel was set on stand and kept undisturbed for 3-5 minutes. Then the clear part of the solution from the bottom of the separatory funnel was collected in vial which was then centrifuged at 1200rpm for 5 minutes (Laboratory Centrifuges, Siga-3K30, Germany). After centrifuge, supernatant was collected for injection.

The extracts were then placed to analysis by GC-2010 equipped with FTD (Flame Thermoionized Detector- Shimadzu). The capillary column was ATM-1, length 30m, ID 0.25mm was used and the film thickness was 0.25μm. Helium was used as carrier gas. The following parameters were used for detection and quantification of ethefon with GC-FTD.

Injection Port SPL

Injection Mode	: Split
Temperature	: 200°C
Flow Control Mode	: Velocity Linear
Purge Flow	: 3.0 ml/min

Column Oven

Initial Temperature : 150°C

Equilibration Time : 3.0 min

Detector Channel 1 FTD

Temperature : 220°C

Stop Time : 8.00 min

Current : 1.00pA

Makeup Flow : 27.5 ml/min

H2 Flow : 1.5 ml/min

Air Flow : 145.0 ml/min

Prior to the injection of the sample extract, standard solutions of ethefon (in different concentrations) were prepared and injected to the above mentioned instrument. The samples were calibrated (retention time, peak area etc.) against three or four pointed calibration curve (0.1 ppm, 0.2 ppm, 0.5 ppm and 1.00 ppm) of standard solution of ethefon. Results were expressed in ppm by the GC software which represents the concentration of the final injected volume. From this value the actual amount of ethefon residue present in the sample was determined by using the following formula (Rahman *et al*., 2011).

Residue in sample (ppm)

$$= \frac{\text{Conc. obtained in injected volume (ppm)} \times \text{Quantity of final volume(L)}}{\text{Amount of sample taken (kg)}}$$

Results and Discussion

Calcium Carbide Measurement (Atomic Absorption Method)

Naturally ripened fruits sequentially mango, banana, papaya and jackfruit are contained Calcium Hydroxide 10.17mg to 12.67 mg, 6.73mg to 6.98 mg, 10 mg to 12 mg and 20 mg to 22mg respectively, (BCSIR, 2011).

Table 1 Presence of the calcium hydroxide in mango samples.

Sl. No	Local markets	Amount of $Ca(OH)_2$ (mg %)		
		Jessore market	**Khulna market**	**Satkhira market**
1.	1	**20.35*d**	10.23f	**20.39*d**
2.	2	**28.44*a**	**20.44*e**	12.39e
3.	3	**26.20*b**	**26.35*b**	12.10e
4.	4	**22.34*c**	**24.24*c**	**28.21*a**
5.	5	**12.82*e**	06.59g	**26.21*b**
6.	6	07.16f	**28.22*a**	**22.35*c**
7.	7	**20.36*d**	**20.15*c**	**20.30*d**
8.	8	**28.20*a**	10.26f	**22.08*c**
9.	9	**26.47*b**	**22.20*d**	10.18f
10.	10	12.32e	**27.85*a**	**26.45*b**
Level of significance		**	**	**

**= Significant at 1% level, ● naturally ripened mangoes are contained Calcium Hydroxide 10.17mg-12.67 mg and ● Calcium Hydroxide present/100gm fruit.

a. In Jessore market among the 10 mango samples 8 mangoes contained Calcium Carbide and from Khulna market among the 10 mangoes 7 mangoes contained Calcium Carbide and from Satkhira market among the 10 mangoes 7 mangoes contained Calcium Carbide (Table 1). Similar results were found by Kanti and Begum (2011). They reported that calcium carbide was found in banana samples from Dhaka markets (Fokirapul, Mirpur and Karwanbazar) and the level was 9.91mg-10.88mg which was over the acceptable level (6.73mg-6.98mg).

Table 2 Presence of the calcium hydroxide in banana samples.

Sl. no.	Local markets	Amount of $Ca(OH)_2$ (mg.%)		
		Jessore market	Khulna market	Satkhira market
1.	1	**10.62*b**	**10.21*a**	06.33e
2.	2	**12.07*a**	05.50e	**10.57*b**
3.	3	04.75h	**08.21*b**	**12.13*a**
4.	4	04.87h	06.21d	**07.43*d**
5.	5	06.65e	**10.37*a**	06.17e
6.	6	06.30g	06.17d	06.19e
7.	7	06.35fg	**07.46*c**	**08.10*c**
8.	8	**07.03*d**	**08.12*b**	**08.50*c**
9.	9	**08.22*c**	04.47f	**10.48*b**
10.	10	06.60ef	04.21f	05.21f
Level of significance		**	**	**

**= Significant at 1% level, ● naturally ripened banana are contained Calcium Hydroxide 6.73mg-6.98 mg (BCSIR) ● Calcium Hydroxide presents in 100gm fruit.

b. According to table 2, in Jessore market among the 10 banana samples, 4 bananas contained Calcium Carbide (which contained excess amount of calcium hydroxide than the optimum level) and from Khulna market among the 10 bananas, 5 bananas contained Calcium Carbide and from Satkhira market among the 10 bananas, 6 bananas contained Calcium Carbide.

Sl. no.

Table 3 Presence of the calcium hydroxide in papaya samples.

Sl. no.	Local markets	Amount of $Ca(OH)_2$ (mg.%)		
		Jessore market	Khulna market	Satkhira market
1.	1	06.15	10.21	05.65e
2.	2	05.16	06.24	09.16ab
3.	3	06.11	06.14	10.08a
4.	4	07.15	05.14	06.44c
5.	5	06.14	07.92	06.60c
6.	6	10.13	07.86	06.41c

Sl. no.	Local markets	Amount of $Ca(OH)_2$ (mg.%)		
		Jessore market	Khulna market	Satkhira market
7.	7	10.50	06.14	09.91a
8.	8	10.49	06.63	08.55b
9.	9	10.21	05.43	09.24ab
10.	10	11.09	05.25	10.20a
Level of significance		**	**	**

**= Significant at 1% level, ● Naturally ripened papayas are contained Calcium Hydroxide 10.17mg-12.67 mg, ● Calcium Hydroxide present/100gm fruit.

c. Table 3 revealed that among the 10 papaya samples from Jessore, Khulna and Satkhira markets none of them contained Calcium Carbide.

Table 4 Presence of the calcium hydroxide in jackfruit samples.

Sl. no.	Local markets	Amount of $Ca(OH)_2$ (mg %)		
		Jessore market	Khulna market	Satkhira market
1.	1	10.18d	**28.21*a**	21.14ab
2.	2	17.21b	**26.32*a**	16.19cd
3.	3	15.21c	20.49bc	16.18cd
4.	4	21.21a	21.36b	21.24ab
5.	5	20.21a	20.32c	15.27d
6.	6	14.24c	20.38c	16.31cd
7.	7	15.17c	15.25e	21.23ab
8.	8	15.08c	15.48e	20.16b
9.	9	21.17a	16.80d	17.20c
10.	10	20.11a	20.02c	21.39a
Level of significance		**	**	**

**= Significant at 1% level, ● Naturally ripened jackfruits are contained Calcium Hydroxide 20.17mg-22.67 mg and ● Calcium Hydroxide present/100gm fruit.

d. Jessore market among the 10 jackfruit samples none of them contained Calcium Carbide and from Khulna market among the 10 jackfruits, 2 jackfruits contained Calcium Carbide and from Satkhira market among the10 jackfruits none of them contained Calcium Carbide (Table 1).

2. Formalin Identification

a. In Jessore markets, 20% mango, 5% banana and 2% papaya samples contained formalin. In Khulna markets 25% mango, 6% banana and 3% papaya samples contained formalin residue. It was found from Satkhira markets that 15% mango and 3% banana samples contained formalin. From the same markets no formalin was found in any papaya samples. It means that generally papayas were not treated by formalin in those markets.

Table 5 Presence of Formalin in mango, banana and papaya collected from southwestern region of Bangladesh

Samples	Locations (Markets)	Number of samples	Number of samples contained formalin
Mango	Jessore	100	20
Banana	Jessore	100	5
Papaya	Jessore	100	2

Table 6 Presence of Formalin in mango, banana and papaya collected from southwestern region of Bangladesh

Samples	Locations (Markets)	Number of samples	Number of samples contained formalin
Mango	Khulna	100	25
Banana	Khulna	100	6
Papaya	Khulna	100	3

Table 7 Presence of Formalin in mango, banana and papaya collected from southwestern region of Bangladesh

Samples	Locations (Markets)	Number of samples	Number of samples contained formalin
Mango	Satkhira	100	15
Banana	Satkhira	100	3
Papaya	Satkhira	100	0

WHO International Agency for Research on Cancer (IARC) in 1995, Recommended *MRL of formalin : 1.00 ppm, *(MRL- Maximum Residue Limit)

3. Ethefon Determination

a. It was revealed that in Jessore, Khulna and Satkhira markets 80-100% mango, 40-50% banana and 30-40% papaya samples contained ethefon residue. Similar results were also found by Rahman *et al.* (2011). They reported that among the 30 tomatoes 14 samples contained ethefon residue collected from Rajshahi and Gazipur markets. The results were also similar with the findings of Prodhan *et al*. (2009). They stated that among the 21 banana samples 9 contained ehefon residue collected from Bogra and Gazipur markets.

Table 8 Quantity of residue of Ethefon estimated from mango, banana and papaya collected from southwestern region of Bangladesh

Samples	Locations (Markets)	Number of samples	Number of samples contained Ethefon	Ethefon concentration (ppm)
Mango	Jessore	10	10	0.25-0.78
Banana	Jessore	10	4	0.34-0.92
Papaya	Jessore	10	3	0.21-0.62

Table 9 Quantity of residue of Ethefon estimated from mango, banana and papaya collected from southwestern region of Bangladesh

Samples	Locations (Markets)	Number of samples	Number of samples contained Ethefon	Ethefon concentration (ppm)
Mango	Khulna	10	9	0.35-0.72
Banana	Khulna	10	5	0.24-0.62
Papaya	Khulna	10	4	0.31-0.63

Table 10 Quantity of residue of Ethefon estimated from mango, banana and papaya collected from southwestern region of Bangladesh

Samples	Locations (Markets)	Number of samples	Number of samples contained Ethefon	Ethefon concentration (ppm)
Mango	Satkhira	10	8	0.32-0.88
Banana	Satkhira	10	5	0.26-0.72
Papaya	Satkhira	10	3	0.22-0.52

FAO/WHO Codex Alimentarius Commission Recommended *MRL of Ethefon : 2ppm, ** ADI of Ethefon : 0.05 ppm/kg body weight

*(MRL- Maximum Residue Limit), (ADI- Acceptable Daily Intake)

Conclusion

From the above results it was revealed that maximum fruits were treated with chemicals. Maximum fruits were ripening and preserving by applying chemicals. So, we should need more consciousness during buying fruits from those markets.

Acknowledgement

The consumers are at greater risk of taking fresh fruits, so we should need more consciousness during buying fruits and chemicaly treated fruits should be avoided.

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