Indian Journal of Biochemistry & Biophysics
Vol. 56, June 2019, pp. 205-213

Determination of fruit quality of calcium carbide induced ripening in mango (*Mangifera indica* L. cv. Alphonso) by physiological, biochemical, bio-enzymatic and elemental composition analysis (EDX)

Preethi Palpandian[1]*, Haripriya Shanmugam[2], E Alli Rani[3] & GTV Prabu[4]

[1,3]Horticultural College & Research Institute; [2]Department of Nano Science & Technology Tamil Nadu Agricultural University, Coimbatore- 641 003, Tamil Nadu, India

[4]ICAR-Central Institute for Research on Cotton Technology, Mumbai- 400 019, Maharashtra, India

Received 17 May 2018; *revised 20 March 2019*

The aim of the study was to determine the postharvest fruit quality of mango cv. Alphonso treated with the laboratory grade (LG) and commercial grade (CG) Calcium Carbide (CaC_2) at the reported highest acceptable dose, and elemental composition analysis (EDX) results to support the statements for traceability of hazardous trace elements in CaC_2, which can serve as a basis towards developing sensors for identifying CaC_2 treated mangoes through detection of trace elements. Physical, physiological, biochemical and EDX of mango cv. Alphonso harvested from farmers' field of Santur village in Krishnagiri district of Tamil Nadu, India were used for the study. All studied physical characteristics except fruit firmness of CG CaC_2 treated fruits did not correlate to desirable fruit characteristics like total soluble solids (TSS), pH, titrable acidity, total sugars and ascorbic acid. Besides, these parameters were desirable only in control fruits, though a number of days taken to reach fruit consumption stage was relatively more compared to CaC_2 treatment. *In vitro*, free radical scavenging potential of DPPH was comparatively higher in control fruits than CaC_2 treated fruits of both grades. Lab grade (LG) CaC_2 treated fruits were non-significant in modifying physical, physiological and biochemical properties of mango cv. Alphonso except for TSS. However, at the end of the experimental period, CG CaC_2 treated fruits recorded higher TSS than LG CaC_2 treated fruits. Energy Dispersive X-Ray (EDX) results confirmed traceability of health hazardous chemical substances of arsenic (As) and phosphorous (P) in both LG and CG CaC_2 lumps. Calcium carbide when used as an artificial ripening agent was not in contact with the fruit surface, the presence of arsenic and phosphorus were not detected in the EDX spectrum, a novel finding of our study.

Keywords: Alphonso, Biochemical, Calcium carbide, Elemental composition analysis (EDX), Physiological

Mango (*Mangifera indica* L.), also known as the "King of fruits" in India is one of the most acclaimed tropical fruits recognized for its richness in flavor and aroma throughout the world. Being a seasonal fruit, mango is available only during the summer months from 87 commercial mango producing countries in the world. Eastern India and Burma, is the place of origin of mango, has given India a distinct place in the world mango market due to its diverse genetic resources. India is the largest producer of mango in the world, with about 30 mango cultivars under commercial cultivation, of which only a few cultivars have demand in the International market[1]. Among the cultivars in India, mango cv. Alphonso is considered as "King of mangoes" because of its unique flavor, size, shape, keeping quality and superior canning property[2]. Due to improper postharvest practices during harvesting, packaging, and storage approximately 20-25% of fruits are wasted. Major challenges affecting mango trade are short shelf life, high susceptibility to chilling injury, uneven ripening postharvest diseases and consumer demand for improved fruit quality. This wastage can be reduced to some an extent through a proper understanding of fruit ripening using scientific methods[3]. Fruit ripening is a highly coordinated, genetically programmed and an irreversible developmental process involving specific biochemical and physiological changes, in turn dictating fruit quality[4].

Use of artificial ripening agent like ethylene gas at the commercial level is an expensive affair. Hence, mango sellers use a cheaper substitute for ethylene, calcium carbide (CaC_2) which in the presence of moisture releases acetylene gas, a weak analog of ethylene[5]. CaC_2 is a greyish black lump used for

*Correspondence:
E-mail: pppreethifruitscience@gmail.com

artificial induction of ripening process in fruits[6]. But there are statements that CaC_2 treated fruits affect the nervous system in humans causing a burning sensation in the chest and the abdomen, vomiting, nausea, and diarrhea due to the presence of arsenic (As) or phosphorus (P)[7]. If pregnant women consume fruits ripened with CaC_2, their children could develop abnormalities[8]. Chronic exposure to the CaC_2 is carcinogenic and could lead to peptic ulcers were reported[9]. In addition, symptoms of arsenic (As) or phosphorus (P) exposure like diarrhea, thirst, and irritation in the eyes, mouth, nose and throat were also reported from previous studies. Inhalation of acetylene decreased hemoglobin, red blood cells, white blood cells, and premature ventricular contraction[10].

Although the use of CaC_2 for fruit ripening is banned in most countries, CaC_2 is available in the market because of wider application in the field of chemical and steel industries. Thus, in the market the practice of using CaC_2 as a ripening agent in mango is still continuing among fruit sellers in India. Rising food safety concerns warrants researchers to assess the risks associated with the consumption of foods contaminated by pesticides, heavy metals or toxins. Clause 2.3.5 of the Food Safety and Standards (Prohibition and Restrictions on Sales) Regulations, 2011 prohibits the sale of fruits in India that have been artificially ripened by use of acetylene gas (commonly known as carbide gas) produced from CaC_2. However, owing to its rapid action on increasing fruit appeal, commercial usage of CaC_2 is still prevalent[11]. Hence, an investigation was carried out to understand the changes in physical, physiological, biochemical parameters including enzymatic activity and free radical scavenging potential in mango cv. Alphonso fruits treated with the laboratory grade (LG) and commercial grade (CG) CaC_2 at the reported highest acceptable dose of 1 g kg^{-1} for mango fruits[12], and EDX results to support the hypothetical statement of presence of hazardous trace elements in pouched CaC_2 which would serve as a basis towards developing biosensors for detecting CaC_2 treated mangoes.

Materials and Methods

Materials

Physiologically matured uniform fruits of mango cv. Alphonso with 90° fullness of shoulder[21] from 10-15 year old healthy mango trees was harvested from three different orchards of varying size (3 acres, 5 acres and11 acres orchard) from Santur village (11° 12′ N latitude and 77° 27′ E longitude), Krishnagiri district, Tamil Nadu, India. An average of 10 trees from a field was selected and fruits ranged from 40 to 50 per tree were harvested during the fruiting season (second week of April) of mango cv. Alphonso. Crop production techniques recommended by Tamil Nadu Agricultural University located in Coimbatore, India were followed for mango cultivation. Mangoes from each orchard were packed in corrugated fiberboard boxes with paddy straw as cushioning material and transported to the laboratory on the same day of harvest. The fruits were pre-cooled to room temperature of 25.5°C with 51% relative humidity. Pre-cooled mango fruits were graded and sorted for uniform size and weighed for carrying out the experiment.

The experimental setup consisted of three treatments replicated thrice with mango cv. Alphonso namely, control fruits (without CaC_2 treatment), fruits treated with LG CaC_2 (1g kg^{-1} of fruit) and fruits treated with CG CaC_2 (1g kg^{-1} of fruit). A set of 12 fruits constituted individual replication; thereby each treatment lot contained 36 fruits. Three fruits from each replication were selected randomly for periodical observations (0^{th}, 3^{rd}, 6^{th}, and 9^{th} day). Chemicals, reagents, and solvents used in this experiment were either of analytical grade (AR) or American Chemical Society standards (ACS) purchased from SD Fine Chemicals Limited, Bangalore, India, Sigma-Aldrich, Bangalore, India, Sisco Research Laboratory, Mumbai, India. Methanol (AR 99.6%) purchased from QReC, Thailand was used for fruit analysis.

Methods

Physiological parameters

The firmness of the fruit was measured at three different positions (proximal, distal and middle portion) by measuring the force required to puncture the fruit[13] using penetrometer (FG 5000a, Taiwan) and expressed in Newton (N). The Physiological loss in weight (PLW) was calculated as [Initial weight - Final weight/Initial weight] × 100. Shelf life of the fruits was recorded by observing a number of days taken by mango fruits from the first day of storage until it reached the stage, wherein the fruit becomes unfit to consume.

Biochemical characteristics

Estimation of total soluble solids (TSS) was determined using a digital refractometer (PAL 3,

Atago Ltd., Japan) at 25°C temperature and it was expressed in degree Brix (°B). Total sugars present in mango fruits was estimated using Anthrone reagent[16]. The pH of extracted juice was measured using pen type pH meter (pH Testr 20, Oakton, EUTECH) after calibrating with buffer capsules of pH 4, 7 and 9 (Merck Specialties Pvt. Ltd., Mumbai). Titrable acidity of mango fruits was measured by titration method[30]. Ascorbic acid estimation was carried out[14] and expressed as mg 100 g^{-1} fresh pulp. Total chlorophyll content of fruit exocarp and mesocarp was determined[15] and expressed as mg g^{-1} fresh weight. One gram of fruit mesocarp or exocarp was used to estimate carotenoids content according to the protocol[16] and expressed as mg g^{-1} fresh weight.

Bio-enzymatic activity

Pectin methylesterase (PME) was assayed according to the protocol[17] and expressed in unit mg^{-1} protein min^{-1}. The polygalacturonase (PG) enzyme assay was performed as per the method suggested[1] and expressed as unit mg^{-1} protein min^{-1}.

Free radical scavenging potential of ripened mango fruits at the edible fruit stage (6th day of storage) was determined[18]. Radical scavenging activity was calculated from the formula given below:

$$\text{Free radical scavenging activity (\% inhibition)} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Elemental composition analysis

EDX is an analytical technique used for the elemental analysis or chemical characterization of a sample used in conjunction with a Scanning Electron Microscope (SEM) (FEI-Quanta 250, Oregon, USA). The sample was cut into small pieces, freeze-dried and placed on one side of the double-sided adhesive carbon conducting tape. The tape was mounted on the 8 mm diameter aluminum stub and placed inside the sample chamber. The elemental analysis of a sample relies on the interaction of source of X-ray excitation and the sample. When the sample is bombarded by SEM's electron beam, electrons are ejected from the atoms on the sample surface. The resulting electron vacancies are filled by electrons from a higher state and an X-ray is emitted to balance the energy difference between the two electrons states. The number and energy of the X-rays emitted from a specimen can be measured by an energy-dispersive spectrometer. The elemental composition of the specimen can be measured as the energy of the X-ray is characteristic of the difference in energy between the two shells and of the atomic structure of the element from which they were emitted.

Experimental design and statistical data analysis

The experiment was carried out with three treatments and three replications (12 fruits in each replication) in a completely randomized block design. Comparison of mean by Analysis of variance (ANOVA) was analyzed at 5% significance level using Web Agri Stat Package 2.0 developed by ICAR-Central Coastal Agricultural Research Institute, Goa (http://icargoares.in/wasp2.0/index.php).

Results and Discussion

Physical characteristics of the fruit

Fruit firmness phenomena started declining immediately after harvest, during ripening and storage period of the study. LG CaC_2 and CG CaC_2 treated fruits required less puncture force of 34.48 N and 35.00 N respectively, while control fruits required significantly more puncture force of 41.23 N force on the 9th day of storage (Fig. 1). This might be due to wax effect and gradual degradation of insoluble protopectins to soluble pectic acid and pectin in

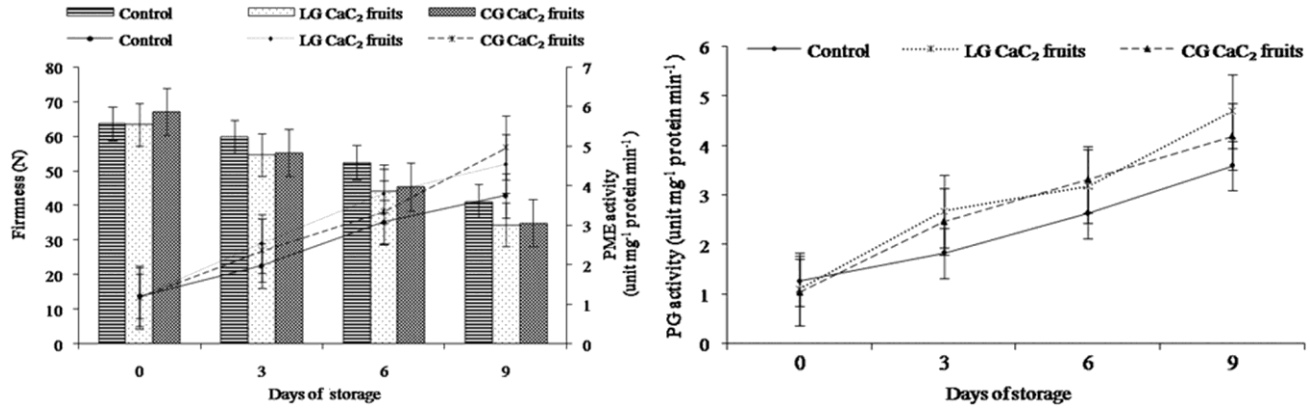

Fig. 1 — Changing pattern in firmness, PME and Polygalacturonase activity in fruit over the period of storage in mango cv. Alphonso

mango cv. Alphonso, thereby delaying softening of the peel in control fruits. In general, a decline in fruit firmness manifested during the time of fruit ripening is not influenced by treatment or cultivar[13, 19].

The physiological loss in weight of 11.40% moisture loss was recorded highest from the fruits treated with CG CaC_2 followed by LG CaC_2 treatment of 10.91%, though not statistically significant (Table 1). As acetylene fumes released by CaC_2 in the presence of water could have resulted in the production of heat and favoured fruit ripening in mango[8]. The shelf life of mango fruits was significantly found to extend till 7.17 days in control fruits followed by CaC_2 treated fruits to 5.83 days (LG CaC_2) and 5.33 days (CG CaC_2). Similarly, Thai mango cvs. Nam Dokmai, Kaew and Chok Anan treated with CaC_2 were prone to mechanical damage due to early ripening[20].

Biochemical fruit characteristics

Organic acids play a major role in respiration and biogenesis of flavor compounds. A slight change in pH during fruit ripening process was observed from the initial 0th day (3.25) to 9th day (4.58) of storage in mango cv. Alphonso (Table 2). The decline in titrable acidity was recorded in all treatments over the period of storage, but LG CaC_2 treated fruits followed by CG CaC_2 treated fruits showed a considerable decline in titrable acidity compared to control fruits. This was in accordance with the previous findings[21]. Ascorbic acid is an important bioactive metabolite and a dietary antioxidant for humans. The ascorbic acid content in both calcium carbide treated fruits of LG (0.15 mg $100g^{-1}$ of fresh pulp) and CG (0.13 mg $100g^{-1}$ of fresh pulp) were comparatively less to that of control fruits with 0.30 mg $100g^{-1}$ of fresh pulp on the 9th day of storage. Ascorbic acid is one of the deciding factors for the fruits desirability on consumption and relatively a fair amount decides the edible quality of the fruits[22].

Total soluble solids were found to be considerable from the 3rd day of storage (10.6°Brix) to the 6th day of storage (16.5°Brix) and then a slight increase in TSS was observed on the 9th day of storage (17.0°Brix) in mango cv. Alphonso (Table 3). CG CaC_2 treated fruits recorded comparatively lesser TSS of 8.5°Brix, 14.5°Brix for the 3rd day and 9th day of ripening respectively, against LG CaC_2 treatment and control fruits. Increase in soluble solids and hydrolysis of starch present in the chloroplast to simple sugar units is an important compositional change that occurs during fruit ripening[23]. This was in agreement with the result obtained from our experiment.

In the case of total sugar, a significant difference among the treated fruits was observed only on the 6th day of storage. Accumulation of total sugars in both CaC_2 treated fruits of LG (7.44%) and CG (7.53%) was found to be less compared to control fruits (10.02%). However, a comparatively higher amount of total sugars (13.97%) was recorded in control fruits, whereas, lower total sugars of 11.06% were observed in CG CaC_2 treated fruits on the 9th day of storage (Table 3). During fruit ripening, regardless of cultivar and treatments activation of starch hydrolyzing enzyme amylase coincided with increasing level of predominant soluble sugars like sucrose, glucose, and fructose[24].

In exocarp of mango cv. Alphonso, until the 3rd day of storage, there was no significant difference in total

Table 1 — Change in moisture content, days to ripe and shelf life of fruits of mango cv. Alphonso during the period of storage

Treatments	PLW (%) (NS)	Days to ripen (days)*	Shelf life (days)*
Control fruits	10.69±0.63	4.33±0.12^a	7.17±0.35^a
LG CaC_2fruits	10.91±0.77	2.66±0.12^b	5.83±0.28^b
CG CaC_2fruits	11.40±0.77	2.00±0.00^b	5.33±0.39^b

NS- Mean value is non significant at $P<0.05$ by LSD.
Mean followed by different letters within a column are significantly different at $P<0.05$ by LSD.

Table 2 — Changes in TSS and Total sugars of calcium carbide treated and untreated fruits of mango cv. Alphonso during the period of storage

Treatments	TSS (°B)				Total sugars (%)			
	0th day (NS)	3rd day*	6th day*	9th day*	0th day (NS)	3rd day (NS)	6thday*	9thday (NS)
Control fruits	7.4±0.05	8.5±0.15^a	13.2±0.07^a	14.6±0.21^a	3.4±0.13	5.1±0.19	10.0±0.28^a	13.9±0.84
LG CaC_2 fruits	7.3±0.13	11.5±0.11^a	18.0±0.04^a	18.7±0.16^a	3.6±0.09	4.2±0.24	7.5±0.37^b	11.5±0.24
CG CaC_2 fruits	7.2±0.06	11.8±0.19^b	18.4±0.13^b	18.1±0.12^b	3.7±0.21	4.1±0.28	7.4±0.42^b	11.1±0.23

NS - Mean is non significant at $P<0.05$ by LSD.
* Mean followed by different letters within a column are significantly different at $P<0.05$ by LS.

Table 3 — Changes in pH, titrable acidity and ascorbic acid content of calcium carbide treated and untreated fruits of mango cv. Alphonso during the period of storage

Treatments	pH				Titrable acidity (%)				Ascorbic acid content (mg 100g^{-1} of pulp)			
	0thday (NS)	3rdday (NS)	6thday (NS)	9thday (NS)	0thday (NS)	3rdday (NS)	6thday	9thday	0thday (NS)	3rdday	6thday	9thday
Control fruits	3.18± 0.05	3.93± 0.02	4.37± 0.04	4.64± 0.02	1.13± 0.01	0.86± 0.01	0.58± 0.01^{a}	0.47± 0.01^{a}	0.84± 0.01	0.71± 0.01^{a}	0.59± 0.02^{a}	0.53± 0.02^{a}
LG CaC_2 fruits	3.32± 0.07	3.82± 0.06	4.63± 0.06	4.59± 0.09	1.12± 0.00	0.82± 0.00	0.4± 0.02^{b}	0.32± 0.00^{b}	0.84± 0.01	0.64± 0.01^{a}	0.32± 0.01^{b}	0.43± 0.01^{b}
CG CaC_2 fruits	3.25± 0.09	4.02± 0.12	4.40± 0.08	4.50± 0.09	1.00± 0.04	0.83± 0.04	0.4± 0.01^{b}	0.33± 0.04^{b}	0.84± 0.01	0.74± 0.01^{b}	0.35± 0.01^{b}	0.45± 0.00^{b}

NS - Mean is non significant at $P<0.05$ by LSD.
Mean followed by different letters within a column are significantly different at $P<0.05$ by LSD.

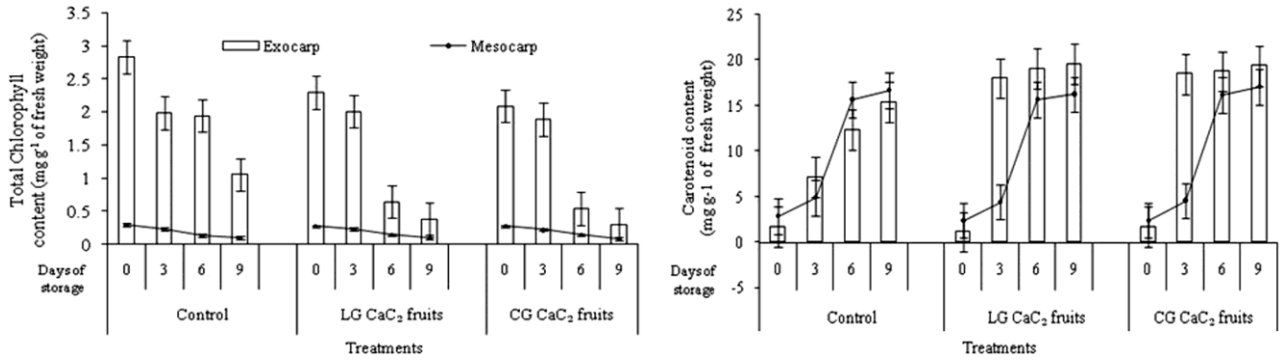

Fig. 2 — Degradation of total chlorophyll and carotenoids in fruit exocarp and mesocarp over the period of storage in mango cv. Alphonso

chlorophyll. Total chlorophyll content present in both CaC_2 treated fruits of LG (0.38 mg g^{-1} of fresh weight) and CG (0.30 mg g^{-1} of fresh weight) were found to be comparatively less to control fruits (1.05 mg g^{-1} of fresh weight). Among the CaC_2 treated fruits, the rate of chlorophyll degradation was greater in CG CaC_2 treated fruits (exocarp 86% and mesocarp 70%) compared to LG CaC_2 treated fruits (exocarp 83% and mesocarp 67%). This increased the spectral appeal of CaC_2 treated fruits, which decides its marketability. The rate of chlorophyll degradation in exocarp and mesocarp tissues of control fruit peel was 63% and 66%, respectively, (Fig. 2).

During fruit ripening process in yellow coloured mango cultivars like Alphonso, degradation of chlorophyll and synthesis of carotenoids or xanthophylls takes place simultaneously[25]. In accordance with chlorophyll degradation, less spectral index ratio of green to yellow (total chlorophyll: carotenoids) is associated with fruit ripening in mango fruits[25].

Carotenoid content gradually increased from initial 0th day (1.21 mg g^{-1} of fresh weight) to 9th day of storage (18.11 mg g^{-1} of fresh weight) in the exocarp of mango cv. Alphonso (Fig. 2), a significant increase was observed only after a 3rd day of fruit storage. Carotenoid content in exocarp was found to be higher in both CaC_2 treated fruits of LG (19.58 mg g^{-1} of fresh weight) and CG (19.37 mg g^{-1} of fresh weight) than to control fruits (15.38 mg g^{-1} of fresh weight) on the 9th day of storage. Degradation of chlorophyll pigments during storage and ripening period is due to conversion of chloroplast to chromoplast, which consists of carotenoids and xanthophylls[26]. In exocarp, carotenoid content increased from initial 0th day (2.54 mg g^{-1} of fresh weight) to 9th day of storage (16.67 mg g^{-1} of fresh weight) in mango cv. Alphonso. However, no significant difference in carotenoid content among the treatments over the period of storage was observed in the fruit mesocarp (Fig 2). On comparing the carotenoid content present between exocarp and mesocarp of the mango fruit in cv. Alphonso, it was noted that initially, carotenoid content of mesocarp (2.54 mg g^{-1} of fresh weight) was comparatively higher than that of exocarp (1.21 mg g^{-1} of fresh weight). But at later stages, carotenoid content was found to be higher in the exocarp

(18.11 mg g^{-1} of fresh weight) than that of mesocarp (16.67 mg g^{-1} of fresh weight) of the fruit.

The Pectin methylesterase enzyme is a de-esterifying enzyme involved in solubilizing cell wall pectins resulting in softening of fruit tissues. Except on the 9th day of storage, there was no significant difference in PME activity over the period of storage. On the 9th day of storage, PME activity in both CaC_2 treated fruits of CG (4.96 unit mg^{-1} protein min^{-1}) and LG (4.55 unit mg^{-1} protein min^{-1}) were comparatively higher than the control fruits (3.75 unit mg^{-1} protein min^{-1}). The results clearly showed that physiologically matured fruits are characterized to increase PME activity during fruit ripening. PME together with PG enzyme accelerated the de-esterification process of pectin during fruit ripening in strawberry[27]. The activity of both PME and PG exhibited a parallel increase in tissue softening and reduction of fruit firmness in mango during fruit ripening (Fig. 1).

Polygalacturonase enzyme has an important role in the modification of cell wall pectin and fruit softening. The activity of PG gradually increased from 1.13 unit mg^{-1} protein min^{-1} from initial 0th day of storage to 4.15 unit mg^{-1} protein min^{-1} on the 9th day of storage in mango cv. Alphonso (Fig. 1). PG enzyme activity was found to be slightly significant among the treatments only from the 3rd day of storage. PG activity was found to be higher in LG CaC_2 treated fruits (4.69 unit mg^{-1} protein min^{-1}) followed by CG CaC_2 treated fruits (4.19 unit mg^{-1} protein min^{-1}) and control fruits (3.59 unit mg^{-1} protein min^{-1}). The rapid rise in PG activity during fruit ripening is due to increased solubilization of the pectin substances resulting in progressive loss of fruit tissue firmness[28].

DPPH radical is one of the most largely employed antioxidant assays for plant samples. Fruit ripening is a sequential oxidative phenomenon that needs a turnover of active oxygen species like hydrogen peroxide and superoxide anion[29]. DPPH radical scavenging activity for CaC_2 treated as well as control fruits were determined on the 6th day of storage, considered as the edible fruit stage for consumption. The DPPH activity of fruit extracts recorded free radical scavenging activity ranged between 11.40% to 13.20% inhibition in mango cv. Alphonso. Free radicals and ROS are generated at the end of the electron transporting chain during respiration in fruits. The DPPH radical scavenging capacity was found to be comparatively higher in control mango fruits

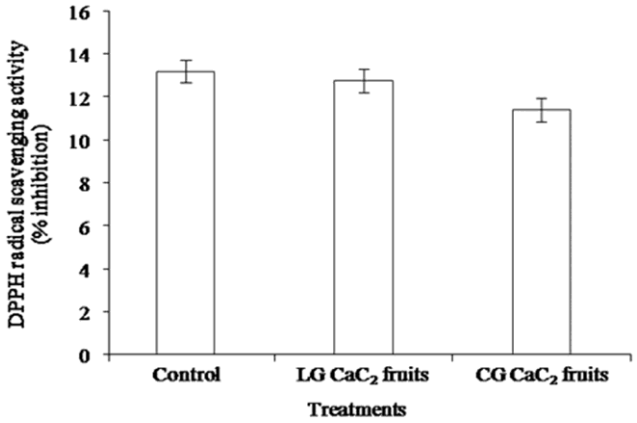

Fig. 3 — DPPH radical scavenging activity observed in fruit mesocarp on 6th day of storage in mango cv. Alphonso

(13.20% inhibition) followed by CaC_2 treated fruits of LG (12.75% inhibition) and CG (11.40% inhibition) (Fig. 3). The higher radical scavenging activity may be attributed to higher levels of dietary antioxidants and provitamin-A content present in control fruits of mango compared to CaC_2 treated fruits at the edible fruit stage (6th day of storage) in mango cv. Alphonso. Antioxidants present in the sample react with DPPH, a nitrogen-centered radical and is converted to 1,1-diphenyl-2-picrylhydrazine because of its electron donating ability[30]. Free radical scavenging activity is highly dependent on the antioxidants present in the fruit extract[31]. Presence of non-enzymatic antioxidants like glutathione, ascorbic acid, and beta carotene could prevent damages caused by reactive oxygen species (ROS) on lipid membrane, nucleic acid and proteins[32]. Mango fruits are a good source of dietary antioxidants like ascorbic acid, carotenoids, and phenolic compounds[33-35].

Elemental composition analysis

Analysis using SEM-EDX was carried out to detect the presence of As and P from the source material of CG and LG CaC_2 lumps as well as treated mango fruits. The spectra of the CG CaC_2 lumps showed nine peaks corresponding to carbon (C), oxygen (O), nickel (Ni), zinc (Zn), arsenic (As), silicon (Si), phosphorous (P), molybdenum (Mo), and calcium (Ca). The CG CaC_2 lumps recorded approximately 0.51 Wt% (Normalized weight percent) of As and 0.12 Wt% of P. However, the spectra of LG CaC_2 lumps showed only six peaks corresponding to C, O, As, Si, P, Mo and Ca. It was observed that the LG CaC_2 used in this experiment is devoid of Ni and Zn impurities. Further, the content of impurities in the CG CaC_2 was four-fold higher than LG CaC_2 lumps (Fig. 4).

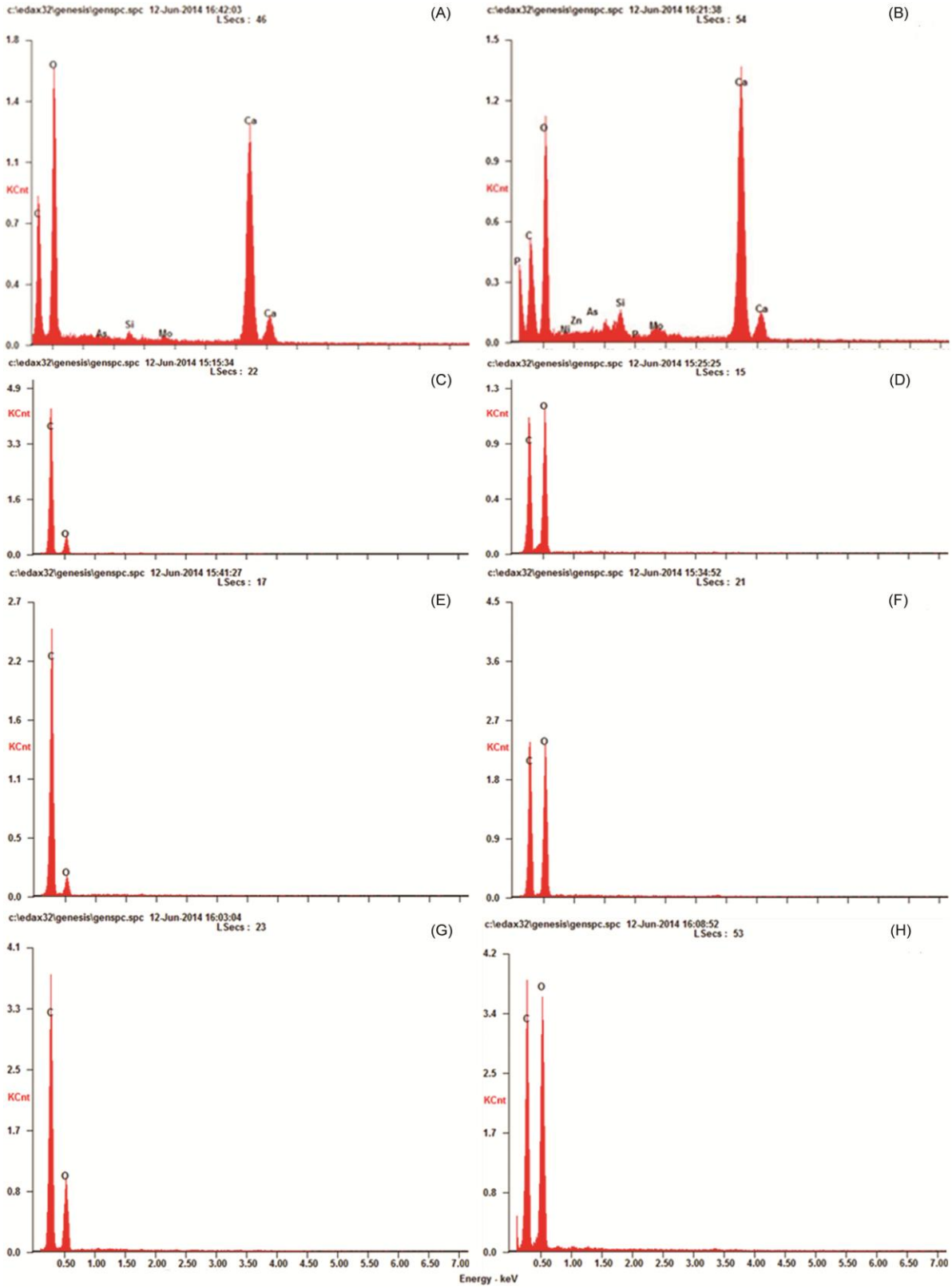

Fig. 4 — EDX spectrum of (A) laboratory grade CaC2; (B) commercial grade CaC2; (C) exocarp; (D) mesocarp of control mango fruits cv. Alphonso; (E) exocarp; (F) mesocarp of LG CaC2 treated mango fruits cv. Alphonso; (G) exocarp; and (H) mesocarp of CG CaC2 treated mango fruits cv. Alphonso

The EDX spectrum of control fruits, LG CaC_2, and CG CaC_2 treated fruits showed only two peaks both in exocarp and mesocarp, which indicated the presence of C and O at various proportions (Fig. 4). Components like As, Si, P, Mo and Ca were not observed in mango fruits treated with both the grades of CaC_2, even though it was recorded in LG and CG CaC_2 source chemicals. There are reports that CaC_2 treated fruits containing traces of As and P are hazardous to human health. But, our experimental results displayed no traces of these elements in CaC_2 treated fruits. The presence of traces of As and P in CaC_2 treated fruits may be detected when the mango fruit surfaces had direct contact with the impurities containing lumps of CaC_2. Since the fruits were prevented from having direct contact with CaC_2 by wrapping with filter paper and placing paddy straw in between them, the presence of harmful constituents in the fruit were absent.

Conclusion

Our experimental results suggest that mango fruits cv. Alphonso exposed to acetylene fumes of LG and CG CaC_2 had downturned fruit firmness, PLW, shelf life, total sugars, pH, titrable acidity, free radical scavenging ability and upsurged bioenzymatic activity of PME and PG compared to control fruits except for TSS. Most importantly, a marked significant difference in the spectral index ratio of green (total chlorophyll): yellow (carotenoids) colour found on fruit exocarpic region (0.30:19.37) had inverse effect on changeover of mesocarpic region of the fruits treated with CG followed by LG (0.38:19.58) and control (1.05:15.38) fruits. The EDX analysis revealed that the traces of hazardous elements (As and P) were present in both the chemical source of LG and CG CaC_2. Hazardous elements were not detected in the exocarp or mesocarp of fruits treated with CaC_2 when CaC_2 lumps were not in contact with the fruit surface of mango. However, control fruits scored higher overall acceptability based on ascorbic acid content as well as free radical scavenging ability in mango cv. Alphonso. Despite the ban on using CaC_2 as an artificial ripening agent in climacteric fruits, the practice is still persistent in many developing countries. Thus this experiment evolves with the proper way of using CaC_2 as an artificial fruit ripening agent. Data generated here could be utilized towards developing biosensors with high sensitivity to distinguish mango fruits treated with CaC_2 even in trace quantities.

Acknowledgment

The research project was funded by the Indian Council of Agricultural Research-National Agricultural Science Fund, India. We express our sincere thanks to Dr. Sivakumar Uthandi, Professor (Agricultural Microbiology) and Dr. K. Soorianathasundaram, Professor (Horticulture) for their kind support rendered towards carrying out this work.

References

1 Rymbai H, Srivastav M, Sharma RR & Singh SK, Lenticels on mango fruit: Origin, development, discoloration and prevention of their discoloration. *Sci Hortic*, 135 (2012) 164.

2 Magallanes-Cedeño R, *Area-wide assessment of the* '*Ataulfo*' *mango cultivation in the Soconusco region of chiapas, Mexico*, (VII International Mango Symposium, Recief city, Brazil) 22, 2002.

3 Srivastava S, Singh KR, Pathak G, Goel R, Asif MH, Aniruddha PS & Vidhu SA, Comparative transcriptome analysis of unripe and mid-ripe fruit of *Mangifera indica* (var. "Dashehari") unravels ripening associated genes. *Sci Rep*, 6 (2016) 325.

4 Giovannoni J, Molecular biology of fruit maturation and ripening. *Annu Rev Plant Physiol Plant Mol Biol*, 52 (2001) 725.

5 Ranganna S, In: *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*, (Ed. by S Ranganna; Tata McGraw-Hill Publishing Company, New Delhi, India) 1986.

6 Asif M, Physico-chemical properties and toxic effect of fruit-ripening agent calcium carbide. *Ann Trop Med Public Health*, 5 (2012) 150.

7 Subramanian S, *Fruit ripening: How do raw mangoes and bananas become ripe when treated with chemicals (Answer-1).* The Hindu. Retrived June, 2015.

8 Kader AA, *Postharvest Technology and biology: an overview. In: Postharvest Technology of horticultural crops 3rd ed*, (Ed. AA Kader; University of California, California: ANR Publications.) 2002, 535.

9 Medlicott AP & Thompson AK, Analysis of sugars and organic acids in ripening mango fruits (*Mangifera indica* L. var. Keitt) by high performance liquid chromatography. *J Sci Food Agric*, 36 (1985) 561.

10 Dhembare AJ, Bitter truth about fruit with reference to artificial ripener. *Arch App Sci Res*, 5 (2013) 45.

11 Arsey RV & Paul RP, Response of mango varieties to exogenous ethylene for fruit ripening. ICAR-News letter, October to December, 2016, 6.

12 Amarakoon RH, Illeperuma DCK & Sarananda K, Effect of calcium carbide treatment on ripening and quality of Velleicolomban and Willard mangoes. *Trop Agric Res*, 11 (1999) 54.

13 Jha SN, Jaiswal P, Narsaiah K, Kaur PP, Singh AK & Kumar R, Textural properties of mango cultivars during ripening. *J Food Sci Technol*, 50 (2013) 1047.

14 AOAC, In: *Official methods of analysis of the association of official analytical chemists 15th ed*, (Association of Official Analytical Chemists, Arlington) 1990, 1058.

15 Yoshida SDA, Forno J, Cock H & Gomez KA, In: *Laboratory manual for physiological studies of rice* (International Rice Research Institute. Los. Banos, Phillippines) 1972, 70.

16 Jensen A, Chlorophylls and carotenoids In: *Handbook of phytological methods*, (Ed. by A Hellebust & JS Crargie; London: Cambridge University Press) 1978, 59.

17 Hagerman AE & Austin PJ, Continuous spectrophotometric assay for plant pectin methyl esterase. *J Agric Food Chem*, 34 (1986) 440.

18 Hou WC, Chen YC, Chen HJ, Lin YH, Yang LL & Lee MH, Antioxidant activities of trypsin inhibitor, a 33 KDa root storage protein of sweet potato (*Ipomoea batatas* L.) Lam cv. Tainong 57. *J Agric Food Chem*, 49 (2001) 2978.

19 Ellong EN, Adenet S & Rochefort K, Physicochemical, Nutritional, Organoleptic Characteristics and Food Applications of Four Mango (*Mangifera indica* L.) Varieties. *Food Nutr Sci*, 6 (2015) 242.

20 Mahayothee B, Neidhart S, Mühlbauer W & Carle R, Effects of calcium carbide and 2-chloroethylphosphonic acid on fruit quality of Thai mangoes under various postharvest ripening regimes. *Eur J Hortic Sci*, (2007) 171.

21 Appiah F, Kumah P & Idun I, Effect of ripening stage on composition, sensory qualities and acceptability of Keitt mango (*Manqifera indica* L.) chips. *Afr J Food Agric Nutr Dev*, 11 (2011) 5096.

22 Sogi DS, Siddiq M, Roidoung S & Dolan KD, Total phenolics, carotenoids, ascorbic acid, and antioxidant properties of fresh-cut mango (*Manqifera indica* L., cv. Tommy Atkin) as affected by infrared heat treatment. *J Food Sci*, 77 (2012) 1197.

23 Hossain F, Parvez AK, Munshi MK, Khalil I & Huque R, Postharvest treatments of radiation and chemical on organoleptic and biochemical properties of mango (*Mangifera indica* L.) in relation to delay ripening. *Am Eurasian J Agric Environ Sci*, 14 (2014) 555.

24 Bretch JK, Yahia EM, Postharvest physiology: Compositional changes during Fruit maturation and ripening. In: *The Mango: Botany, production and uses 2nd ed*, (Ed. RE Litz; Cab International) 2009, 484.

25 Gill PS, Jawandha SK, Kaur N & Verma A, Changes in fruit colour of dusehari mangoes during ethephon induced ripening. *Int J Agric Environ Biotech*, 8 (2015) 97.

26 de Jesus OPJ, Yahia EM & Gardea AA, Changes in external and internal color during postharvest ripening of 'Manila'and 'Ataulfo'mango fruit and relationship with carotenoid content determined by liquid chromatography–APcI+-time-of-flight mass spectrometry. *Postharvest Biol Technol*, (2008) 145.

27 Osorio S, Bombarely A, Giavalisco P, Usadel B, Stephens C, Aragüe, I, Medina-Escobar N, Botell MA, Fernie AR & Valpuesta V, Demethylation of oligogalacturonides by FaPE1 in the fruits of the wild strawberry *Fragaria vesca* triggers metabolic and transcriptional changes associated with defence and development of the fruit. *J Exp Bot*, (2011). 465.

28 Fischer RL & Bennett AB, Role of cell wall hydrolases in fruit ripening. *Annu Rev Plant Physiol Plant Mol Biol*, 42 (1991) 675.

29 Jimenez A, Creissen G, Kular B, Firmin J, Robinson S, Verhoeyen M & Mullineaux P, Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta*, 214 (2002) 751.

30 Huang D, Ou B & Prior RL, The chemistry behind antioxidant capacity assays. *J Agric Food Chem*, 53 (2005) 1841.

31 Saini P & Gangwar M, Enzyme and free radical inhibitory potentials of ethyl acetate extract of endophytic actinomycete from Syzygium cumini. *Indian J Biochem Biophys*, 54 (2017) 207.

32 Kang HM & Saltveit ME, Antioxidant capacity of lettuce leaf tissue increases after wounding. *J Agric Food Chem*, 50 (2002) 7536.

33 Ma X, Wu H, Liu L, Yao Q, Wang S, Zhan R, Xing S & Zhou Y, Polyphenolic compounds and antioxidant properties in mango fruits. *Sci Hortic*, 129 (2011) 102.

34 Reddy SVR, Sharma RR, Srivastava M & Kaur C, Effect of pre-harvest application of salicylic acid on postharvest behavior of 'Amrapali' mango fruits during storage. *Indian J Hortic*, 73 (2016) 405.

35 Preethi P, Soorianathasundaram K, Sadasakthi A, Subramanian KS, Paliyath G & Subramanian J, Influence of Hexanal formulation on storage life and post-harvest quality of mango fruits. *J Environ Biol*, 39 (2018) 1006.