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ANALYSIS OF ORGANOLEPTIC PARAMETERS AND HEAVY METALS IN ARTIFICIALLY RIPENED MANGO FRUITS IN PAKISTAN.

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ABSTRACT

Calcium carbide (CaC_2)is still commonly used as artificial fruit ripening agent for mangoes in many developing countries like Pakistan despite of the major concern of arsenic (As) and other metals contaminations. We used Particle Induced X-ray Emission (PIXE) for the detection of arsenic traces in commercially availableCaC_2andartificially ripened mangoes (ARM). Presence of harmful arsenic residues in calcium carbide (CaC_2) treated fruits provided evidences that arsenic traces were transferred from calcium carbide (CaC_2) which is used for ripening. Mature green mangoes were treated with CaC_2in three different ways (T_1- T_3) and were compared to fully ripened mangoes bought from local markets (T_4) and non-treated mangoes (T_0). Pulverized mango samples were irradiated by 3MeV collimated protons from 5MV tandem accelerator at National Institute of Physics, Pakistan and emitted X-ray spectrum was analyzed using GUPIXWIN to detect significant amount of As traces with differential presence of several other elements. Arsenic presence was further validated and endorsed in mango fruits using Graphite Furnace Atomic Absorption Spectrometry (GFAAS) and Inductivity Coupled Plasma Mass Spectrometry (ICP-MS). Absence of As traces in non-treated control mangoes has provided evidence of As contamination in artificially ripened mangoes is associated with CaC_2 used for ripening. Arsenic residues in CaC_2 treated mangoes can easily added up to already available arsenic exposure towards the limits shown for several cancerous diseases. Present study will not only provide a direct method of arsenic detection in fruits, but also suggest the need of strict implementations and improvements in the existing food safety rules and regulations to completely ban this carcinogenic chemical for its future applications.

Key words: PIXE; Arsenic; Hazardous; Calcium Carbide; Post harvest.

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INTRODUCTION

Mango *(Mangifera indica L.)* is a climacteric fruit and commonly considered as "king of fruits" due to its excellent flavor, color, nutritious quality, attractive fragrance, and most importantly due to delicious taste. Pakistani mangoes rank good worldwide due to ~139 kt production and ~7.6% share in the world market. In Pakistan almost 250 different mango varieties are found, while the most important commercial cultivars are Anwar Ratul, Dasehri, Langra, Sindhri, Chaunsa, Maldha and Fajri. Mango fruits usually mature in 100-150 days after flowering and then the fruit can possess its best flavor and aroma (depending upon the variety) if allowed to ripen on trees. In Pakistan, most of the mangoes are harvested at mature green stage and transferred to local markets, where ripening process is taken care by application of different chemicals before marketing. Mango fruit is extremely perishable to transport when fully ripened thus requires appropriate postharvest technologies for fruit ripening. Conventional fruit transportation methods and irregular storage are the main cause of more than 30-40% fruits lossin developing countries (Carrillo and Mariotti, 2000; Narayana *et al*., 1996; Shahbaz *et al*., 2009). Various artificial methods of fruit ripening have been observed mostly to meet consumers' demand and other economic factors. Different fruit postharvest practices such as hot water treatment, cold treatments, ethaphon, calcium chloride (Mahmood *et al*., 2013; Rahman *et al*., 2016; Gandhi *et al*., 2016) ethylene, and methyl jasmonate can be used. Ethylene, and methyl jasmonate are non-toxic ripening agents but relatively expensive.

Heavy metals are reported among the major contaminants of food supply and mainly considered as the most important form of environmental pollution (Zaidi *et al*., 2005; Khan *et al*., 2008). Heavy metals are hazardous contaminants in food and environment due to their non-biodegradable nature and continuous intake of heavy metals is of concern worldwide. According to the World Health Organization (WHO 1995), heavy metals must be controlled in food sources in order to assure public health safety. Excessive concentration of food heavy metals are found associated with the etiology of a

number of diseases, especially cancer, cardiovascular, renal, neurological, and bone diseases (Chailapakul *et al*., 2008; Järup, 2003;Radwan and Salama, 2006;Beccaloni *et al*., 2013; Barone *et al*., 2015; Jackson and Punshon, 2015).

The popularity of CaC_2 is based on its easy application,cost-effectiveness, rapid fruit ripening, help indirectly in fruit shelf life/transport, and mainly protects the texture, color and sweetness of the fruits for better marketing. On exposure to moisture, CaC_2 produces acetylene gas (C_2H_2), which enhances heat and fruit ripening similar to ethylene (C_2H_4). CaC_2 is produced in an electric arc furnace industrially by heating lime and coke mixture at ~2000°C.Commercially available CaC_2 is not pure, as it always contains calcium arsenide (Ca_3As_2) and calcium phosphide (Ca_3P_2), which in presence of humidity changes to fat soluble arsine (AsH_3) andphosphene (PH_3) respectivelyfollowed by rapid diffusioninto the fruit skin and then deposits in the fruit pulp causing extremely hazardous effects on human health (Asif, 2012). Daily intakes of arsenic can cause diarrhea, irritation of digestive system, stomach cancer and liver failure. Free radicals from carbide play a major role in the ageing process as well as in the onset of cancers, heart disease, stroke, arthritis and perhaps different type of allergies (Essien *et al*., 2018).

Several analytical techniques have been recently used to determine the elemental composition of contaminated food samples, but usually due to complexity of sample preparation handling and additional steps can reduces the reliability of thepresence of particular element in the given samples. Advent and improvement of technologies such as, Proton Induced X-ray Emission (PIXE), Graphite Furnace Atomic Absorption (coupling with cold vapor or hydride generation), Inductivity Coupled Plasma Optical Emission Spectrophotometer (ICP-OES), and Inductivity Coupled Plasma Mass Spectrometry (ICP-MS) made it easy to determine the quantities for any hazardous or beneficial elemental in the given samples including food (Haruyama and Saito, 1995; Flocchini *et al*., 1972; Zaidi *et al*., 2005; Radwan and Salama 2006; Naser *et al*. 2009; Jackson and Punshon 2015). Usually trace elements in food are in low concentrations, so we need really effective, robust, precise, accurate and sensitive method (Jackson and Punshon, 2015). Atomic and nuclear technologies has been very extensively utilized due adequate sensitivity to measure a wide range of elements in life sciences (Wang and Nastasi, 1995;Johansson and Campbell, 1988; Johansson. 1989;Naser *et al*., 2009; Bertrand *et al*., 2003) such as PIXE (Wang and Nastasi, 1995; Haruyama and Saito, 1995; da Silva *et al*. 2011; Stihi *et al*. 2008), and X-ray fluorescence (XRF) (Zaidi *et al*., 2005; Wang and Nastasi. 1995). PIXE can reliably, detect the presence of essential and toxic metals in parts per million (Johansson and Campbell, 1988; Flocchini *et al*., 1972; Pantelica *et al*., 2011; Kamal *et al*., 2007). Every method has its own pros and cons for different elements, therefore the use of more than one method can be helpful in term of increased the reliability and reduced the biasness in the analyzed results.

This study was aimed to check the presence of arsenictraces in the most favorite Pakistani mango variety "Langra" treated with CaC_2. Weused a pragmatic approach for identification and quantification of inorganic As in mango samples using three different state of art technologies including, PIXE (at National Center of Physics, PK), GFAAS (at Pakistan Institute of Nuclear Science and Technology, PK) and ICP-MS (Dartmouth Trace Element Analysis Core Facility, Dartmouth College, USA). Fully ripened, ready to eat mangoes of the same variety were bought from the local markets and super markets of Islamabad, Pakistan. We included these mangoes in this comparative investigation to confirm the arsenic contamination in the mangoes.

MATERIALS AND METHODS

Fruit Sampling and Post-Harvest Treatments: Randomly selected un-ripened, green mangoes (*cv* sindhri) of almost similar age were collected directly from the orchids located in district Multan(Province Punjab, Pakistan) and artificially ripened with CaC_2. Fully ripened, ready to eat mangoes were bought from two different local markets including a small fruit shop and supper store (Islamabad, Pakistan). All of the fruits were divided into five different groupsto check the presence of As due to CaC_2treatment in three replicates as, Group-1(T_0): Control mango fruits were kept inside the papers (used newspapers) for 4-6 days in a corrugated wooden container (dimension 7″ x 12″ x 26″) without any chemical treatment and this group will be used as control for comparisons. Group 2 (T_1): Mango fruits were treated with CaC_2similar to the method used in our markets by placing CaC_2 in an envelope in the corner of wooden boxes containing fruits and kept at 27 ± 4°C for 4-6 days.While, Group-3 (T_2): Mangoes were initially immersed in 5% solution of CaC_2 for 10 minutesand then stored for 4-6 days in wooden box as described above.Group-4 (T_3): CaC_2 powder was directly applied onto fruit surface for 10min and then fruits were stored for4-6 days in a wooden box like above. Group-5: T_4: Fully ripened, ready to eat mango fruits of the selected variety were bought from local fruit markets.

Organoleptic Parameters: We measured the fruit aroma, and flavor of all the treated and control fruit samples as described by Hedonic scale (Larmond 1987). Critical judgment on the given Performa by a panel of 20 experts (30–50 years old) onrandomly cutmangoes into 6-7 piecesfor color, aroma and flavor. Peel colour of

mangoes included in T_2-T_4 was compared to control untreated mangoes (T_0).

Mango Sample Preparations: Fully ripened fruits were peeled off and then samples were cut into small pieces with the help of uncontaminated steel knife. The samples were dried in an oven at 60 ˚C for 48 hrs to remove all moisture present in peel and pulp. Dried samples were finally ground to make homogeneous powder using pre cleaned mortar and pestle.

Approximately 0.6 g of powder sample was weighed carefully by electronic balance and pelletized using Laboratory Hydraulic Press (Carver USA; mode: 4350, LC; Serial No. 4160505). Hydraulic press of 24000 psi was used to make pellets of 2 mm thickness and 13 mm diameter. Two pellets were formed from each sample. The pellets were placed in desiccators to avoid moisture and any environmental contamination. The pellets were also put under lamp for some time before PIXE analysis to get rid of any moisture because vacuum problem is created in chamber during analysis.

Elemental Analysis of Mango Fruits and Instrumentation: We examined and analyzed the edible part, pulp of mango fruits using,

PIXE Analysis: Ground fruit samples were compressed manually using Laboratory Hydraulic Press (Carver, USA; Model: 4350.LC; Serial No. 4160505) under pressure of 22000 Pounds/10 Metric Tons to make 13 mm pallets. Fruit pallets were irradiated with proton beam of 3MeV using 5MV Pelletron Tandem Accelerator at Experimental Physics Laboratory, National Centre for Physics, Islamabad. The collimated beam of size approximately 2 mm size was used to irradiate the samples in vacuum chamber. During irradiation, the beam current was in the range of 10 to 20 nA. The X-rays emitted in this process were measured by SDD with high resolution (FWHM 160 eV at 5.9 keV Mn Kα energy). The detector was placed at an angle of 45o to incident beam. The advantage of 45° orientation is the maximum characteristic X-rays collection and minimum background radiations. The distance between detector and target was 6 cm. Mylar absorber of 100 μm with no hole was used between target and detector to reduce low energy background. The vacuum inside the scattering chamber was 10-6 torr.

To check the accuracy of experimental result NIST Standard reference material apple leaves (SRM 1515) was used. For quantitative analysis of sample GUPIXWIN software package was used. GUPIXWIN software package is used to convert spectral data into elemental concentration. GUPIXWIN software analyzes the spectrum for elements and the data finally are transformed to Microsoft Excel format where final graphs are designed.

Different elements present in all samples of fruits were identified and estimated their concentration by using this software package. The concentration of each element in fruits were obtained by analysing the data using HED files and satisfying the fit requirements. Similar spectrum of SRM is obtained by applying same process. The different peaks of spectrum relate to different elements in the sample. The region under peak corresponds to the concentration of specific element in sample.

GFAAS: Arsenic (As) analysis was carried out by graphite furnace atomic absorption spectroscopy (GFAAS) using a Perkin Elmer 5100/HGA 600 instrument at Pakistan institute of Nuclear Sciences and Technology (PINSTECH). The method parameters were previously optimized by establishing ash and atomization curves for each metal. Wet digestion of all the ground mango samples was done with HNO_3 (65%) followed by heating at 70°C for one hour. $HClO_4$ (70%) was added after cooling at room temperature and prolonged heating at 240°C was done to remove all the white fumes (Fatima and Rahman, 2009). Finally, the clear solution was analyzed after required dilution. We used Hitachi model Z-2000 polarized Zeeman Atomic Absorption Spectrophotometer for this study using dual modes of atomization, graphite furnace AAS (GFAAS) and flame AAS (FAAS) as routinely used at PINSTECH, Islamabad, Pakistan. The elemental measurements were further validated using standard reference materials (SRM) analysis taken from National Institute of Standards & Technology (NIST).

ICP-MS: Almost 100 mg of dried mango powder from all the samples was used to digest by using 2mls of HNO_3 and heated to 180°C for 20 minutes. Cooled samples were brought up to 15 ml volume using distilled water. Digested mango samples were diluted 100 times with distilled water and analyzed using Agilent 7700x ICP-MS in “He mode analytes” withintegration times ~500 ms-1 sec at central facility of Dartmouth College, NH, USA. Data was analyzed and elemental concentrations were calculated as described (Jackson and Punshon, 2015).

Written Informed Consent was also taken from the participants for this manuscript.

Dietary Intake of Arsenic (DAsI): Daily intake of As in an average adult was calculated by elemental data obtained from different CaC_2 treated as well as non-treated control mango groups. Dietary As intake (DAsI) was assessedas follow,

$$DAsI = C \times Q$$

Where, C is the concentration of As in the selected mangogroup ($\mu g\ g^{-1}$) and Q, daily consumption of mangoes ($g\ kg^{-1}$ of body weight) in 2-3 days (World Health Organization, 2009; EFSA, 2010).

Validations and Statistical Analysis: All the elemental analysis was done in three replicates of all the pooled mango samples in different groups after treatment. For accuracy and reproducibility, certified reference materials were used for the instrumental calibrations. This study was conducted to evaluate the effect of CaC_2 on organoleptic parameters and elemental profiling of mango fruit therefore One-way ANVOA with fixed effects was preferred to identify the statistically significant difference in among different studied groups. Tukey's HSD (honestly significant difference) test was employed for multiple comparison followed by Dunnett's test, Scheffé method and Bonferroni and Holm procedure. Different methods were employed to focus on biologically meaningful results which can be vital in a greater inspection of biologically interesting effects.

For Tukey's HSD, Scheffé method and Bonferroni and Holm procedure, an online calculator was used (https://astatsa.com/OneWay_Anova_with_TukeyHSD/). Dunnett's test was employed by using MS Excel.
Then correlation analysis (using Pearson's correlation) was applied using MS Excel to check the correlation among heavy metals with elemental profile of artificial ripened mangoes.

RESULTS AND DISCUSSION

In this study calcium carbide (CaC_2) was investigated for its arsenic residues. For this purpose we purchased CaC_2 from three different local suppliers. PIXE and GFAAS showed significantly high amount of arsenic traces (~990-1153ppm). Such a high amount of inorganic arsenic can contaminate the fruits when treated with CaC_2 for the purpose of artificial ripening. Arsine (gas) released from contaminated CaC_2 combines with air to form inorganic arsenic compounds, which have toxic and carcinogenic effects due to consumption of such fruits. Consumption of heavy metal contaminated fruits can cause heavy metal toxicity and cancer in Pakistan.

To investigate the presence of Astraces in artificially ripened mango fruits due to commonly used postharvest treatment of CaC_2 in Pakistani local markets, we treated un-ripened green mangoes with CaC_2 in different ways as shown in the Table-1. We also used a market mimicry treatment method used in our local markets (addition of CaC_2 packets in mango containers and kept for 2-4 days (Group-2). Different treatment groups were compared for their elemental analysis with control (non-treated, Group-1) mango fruits and fully ripened, ready to eat mangoes bought from local markets (Group-4). The effects of CaC_2 on organoleptic properties of mangoes were assessed. As expected the control mangoes showed delayed ripening with green and yellow non-homogenous patches even after 4-5 days (Table-2).The fruit color change is usually result of unmasking of fruit pigments through chlorophyll degradation, anthosyanin synthesis and carotenoids accumulations, in either case of natural or artificial fruit ripening. Similarly, increased metabolism and hydrolysis, decreased fruit acidity, and sugars can bring sweetness(Islam *et al*., 2016). Application of artificial ripening agent can speed up the ripening mechanism when required. The mangoes of Group-2 were fully ripened in 2-3 days with homogeneous attractive yellow color and nice texture that no one can virtually distinguish from naturally ripened fruits. While, application of CaC_2 in treatment Group-3 and 4, where we treated green mangoes only for 10 min initially (Table-1),resulted in non-homogenous greenish yellow color due to short term acetylene gas production in the container which wasnot enough to fully ripe mango fruits but the process was initiated. Fruit ripening by using CaC_2 does not required any technical training so local fruit venders use it in higher quantities to ripen immature fruit. Therefore, neither of our 5% CaC_2 treatment nor direct CaC_2 application for 10 min showed much effect on mango fruit ripening in our experimental conditions.

Fruit ripening is a biochemical process which involves a number of metabolic activities such as enhanced ethylene production and respiration, change in carbohydrates, synthesis of carotenoids, degradation of chlorophyll and conversion of starch into sugars. These activities lead to a series of physiological changes in color, taste, flavor, aroma and texture to consumer's acceptable level. All these factors combine to develop a complete sensory and edible profiles of a fruit (Lalel *et al*., 2003). Among the physiological properties, the skin color of the mango fruits is a vital characteristic for its marketing as it makes the commodity more attractive. Our data showed homogenous yellowcolor of mangoes of the Group-2 (T_1) like market fruits as expected due to almost same effect of CaC_2. The appearance and smoothness of fruit skin is also very important acceding to consumers view point. Mangoes of Group-2 were perfectly ripened and attractive in comparisons to control group (shriveled and un-ripened patches on peel).

Anjum and Ali (2004) conducted a study to investigate the effects of different calcium salts (calcium chloride, calcium sulphate and calcium ammonium nitrate) on organoleptic parameters and they concluded that these salts delayed the ripening process of mangoes (cv. Kala Chaunsa) and positively influenced the skin and pulp colour but badly affected the aroma, flavour and taste of mangoes and hence resulted in bad/poor eating quality. Another similar study was conducted by Mehmud *et al*. (2015) and they used calcium carbide along with calcium chloride, calcium sulphate and calcium ammonium nitrate to investigate organoleptic parameters of Lokhna, Himsagor, Gopalvogh and Langra varieties of mangoes and they concluded that CaC_2 hastened the ripening process and improved the texture,

aroma and taste of mangoes. Padmini and Prabha (1997) reported that mangoes fruits treated with CaC_2 attained attractive yellow colour, because of high carotenoids content, earlier than untreated control group. Our results corroborate with Padmini and Prabha (1997) and Mehmud *et al*. (2015).

We also observed effect of CaC_2 and its toxicity by difference in color of dried ground powder of mango pulp and peel samples of all the treated groups with the control (non-treated) group of mangoes. Dried peel and pulp powder of mangoes included in control group retained their distinct orange-yellow color while toxic effects of CaC_2 has turned dried peel and pulp powders of mangoes included in T1-T4 into dark brown or blackish-brown color (Fig-1 A & B).We analyzed the elemental concentrations of only mango pulp due to, i) Pulp is the main edible part of the fruit, ii) Peel pallets were very brittle and not compact enough to proceed with PIXE analysis. Proton beam irradiated hydraulically pressed pallets of all the treatmentsand control fruit groups are given in Fig-1C.

Quantitative analysis of all essential as well as the trace elements of different groups of mango fruits were done with PIXE and concentration of elements in parts per million (ppm) including sulfur (S), potassium (K), calcium (Ca), iron (I), manganese (Mn), nickel (N), copper (Cu), zinc (Zn) and arsenic (As) were determined from spectrum obtained with 3MeV proton beam followed by analysis using GUPIXWIN software. Presence of peak for arsenic (10.543 keV) in PIXE spectra of all the CaC2 treated mango samples and absence of peak for arsenic in control samples are evidence of arsenic contaminations in CaC_2 treated mangoes (representative spectra of all the groups are shown in the Fig-1D).For validation purpose, the elemental concentrations of Standard Reference Material (SRM) provided by NIST weremeasured at NCP (National Centre for Physics) Islamabad, Pakistan initially which were close to the certified values (7-10% variations due to different experimental conditions). Our data showed negligible difference inthe elemental concentrations of all the major and minor elements in given treatment groups andcontrol group except the concentration of As (Table-3). Interestingly, no As traces were detected in the control (non-treated) mango pulp samples, while significantly high concentrations of As was found in fully ripened mangoes taken from market (T_4) and ourtreatment T_2(replica of market CaC_2treatment) as shown in Fig.2A.A small amount of arsenic residues were detected in mango fruits treated with CaC_2 for 10 min (T2& T3). Reason for less arsenic detection can be less availability of CaC_2, or arsenic settles down in solution form and provides less contact with in sort time. Our results corroborate with Chandel *et al*. (2017).Absence of arsenic in control mangoes provide evidence that As contamination in the mango fruits is linked to CaC_2. However, different concentrations of As have been found in different treatments of fruits with CaC_2. The highest amount of As was observed in the group-2 mangoes followed by market samples thus confirmed the commercial use of CaC_2 for fruit ripening (Fig 2A)for local use only, as CaC_2 is strictly ban worldwide and cannot be used for export quality fruits due to regular monitoring and quality checks internationally.Therefore alternative methods for postharvest treatments are being used for export quality fruits to sell in global markets.

From correlation matrix it is revealed that arsenic in mangoes pulp has moderate negative correlation with phosphorus P (r^2 = -0.59) and potassium K (r^2 = -0.5) while it showed very strong negative correlation with calcium Ca (r^2 = -0.83)

Data obtained from PIXE clearly revealed that arsenic residue present in CaC_2 treated mangoes were only due to CaC_2 treatment (Figure 3a). We validated PIXE results by GFAAS and ICP-MS.Both methodsalso confirmed the presence of As traces in different groups including market mangoes, though due to different levels of instrumental sensitivities, a direct comparisons of the relative quantifications of As detected in the samples by different methods was not possible (Fig 2). ICP-MS, the most sensitive technique available for such analysis (Jackson and Punshon, 2015) also confirmed the presence of As in all the treatment groups in comparisons to control samples (Fig 2B & C).

Arsenic residues detected in artificially ripened fruits can disturb different body as well as metabolic functions and these arsenic traces can lead to various lethal diseases like cancer(Siddiqui and Dhua, 2010). Therefore, we also calculated daily minimum amount of As intake due to consumption of ~200mg of CaC_2 treated mangoes per day per body weight of ~60 Kg and our PIXE data showed As consumption ~1-9.8µg/kg body weight (bw) per day. Due to its popularity of this fruit and unavailability during off season in local markets, mango consumption increases many folds in Pakistan.Whereas the bench mark dose of 0.3-8 µg/kg body weight (bw) per day of As to cause 1% increased risk of cancer was recently published for adults and even less for kids and toddlers (Gundert-Remy *et al*., 2015). Now a days, major emphasis is on to reduce the arsenic exposure and to make long term measures to reduce the arsenic contaminations in food because of arsenic presence ubiquitously in soil and food chain is already at alarming levels.Recent studiescomprising ~1200 samples of groundwater from Pakistan already showed detailed hazard with risk maps of elevated As contamination in groundwater with thresholds of 10-50 mg/liter and ~50-60 million people are using that groundwater in Lahore and Hyderabad, Pakistan (Podgorski *et al*., 2017). Based on our calculations, the higher rate of mango consumption during season can accumulate dangerous

levels of As in our bodies through CaC_2 treated fruits and is linked to increased percentages of cancerous diseases in Pakistan.

We also examined CaC_2 powder bought from local market which was used to ripen fruits (mangoes) for this study and commercial grade CaC_2 used in the market by PIXE. Enormous amount of arsenic (153 mg/kg) and phosphorus (155 mg/kg) was recorded in these CaC_2 samples.

On daily consumption of such artificially ripened mangoes arsenic may damage different body organs and metabolic functions of the consumers which may lead to various lethal diseases like cancer. We calculated and compared the mean estimated daily intakes (MEDI) of elements detected by PIXE. The provisional tolerable daily intakes (PTDI) recommended by Joint FAO/WHO Expert Committee on Food Additives are 1.5 mg/kg bw for Cr, 0.14 mg/day kg bw for Mn, 0.7 mg/day kg bw for Fe, 0.02 mg/day kg bw for Ni, 0.4 mg/day kg bw for Cu, 0.3 mg/day kg bw for Zn and 0.0003 mg/day kg bw for As. Our results revealed that arsenic detected in CaC_2 ripened mangoes was much higher than the provisional tolerable daily intakes recommended by Joint FAO/WHO Expert Committee on Food Additive (Table 4).

Based onthis report and associated possible health hazards due to artificial fruit ripening agents, there is urgent need of extensive screening of varieties or fruits treated with CaC_2.Some recent studies showed that artificial ripening of fruits results in decreased nutritional values of artificially ripened bananas and pineapples with special reference to vitamin C, proteins and beta-carotene contents along with heavy metal contamination (Hakim *et al*., 2012; Mahmood *et al*., 2013; Zenebe *et al*., 2015; Islam *et al*., 2018; Maduwanti and Marapana, 2019).Regular consumption of such fruits on regular basis causes severe hazards to human health like diarrhea, skin allergy, central nervous system depression, liver and kidney disease, cancer, nausea, gastrointestinal irritation and vomiting (Hakim *et al*., 2012). Almost every country around the globe has food policies and regulations for artificial fruit ripening but implementation is lacking in developing countries like Pakistan, India, Bangladesh etc.(Islam *et al*., 2016).

Table-1. List of mango treatments with CaC_2 in different groups.

Samples	Ids	Mango Treatments
Group-1	T_0	Control non-treated mango samples were kept inside corrugated wooden container (dimension 7″ x 12″ x 26″).
Group-2	T_1	Fruits were treated with CaC_2 to mimic the commercially used method by placing CaC_2 in an envelope in the corner of wooden box.
Group-3	T_2	Mangoes were initially immersed in 5% solution of CaC_2 for 10 minutes following storage for 4-6 days in wooden box.
Group-4	T_3	CaC_2 powder was directly applied onto fruit surface for 10 min followed by 4-6 days storage in a wooden box.
Group-5	T_3	Fully ripened, ready to eat mango fruits of same selected variety were bought directly from local fruit shop and supper market.

Table-2. Organoleptic characteristics of mango fruits.

Parameter	Group 1 T_0	Group 2 T_1	Group 3 T_2	Group 4 T_3	Group 5 T_4
Time required for ripening	5-6 days	2 days	3.5 day	4 day	Bought fully ripened
Color of the skin	Greenish with yellowish patches	Uniform yellow color	Greenish yellow with dark patches	Greenish yellow with dark patches	Uniform yellow color
Flavor, pulp color, aroma	Late ripened, soft pulp and attractive	Fully ripened, nice pulp and very attractive	Same as control but less attractive	Same as control but less attractive	Fully ripened, nice pulp and very attractive
Skin shriveling	Little bit due to delayed ripening	No	Too much shriveled	Shriveled widespread	No

Table 3: Concentrations of Different Elements (in ppm) in Mango Fruit Samples treated with Different Concentrations of CaC_2 and Fruits from Market.Same letters indicate no significant difference . measured by One way ANOVA with post-hoc Tukey HSD test.

PIXE Analysis	CaC_2 Treatments				
Elements	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
Al	8.525 ± 1.73^a	7.175 ± 0.07^a	7.975 ± 0.05	$10.975 \pm 0.88a$	6.03 ± 0.66^a
P	0.147 ± 0.01^a	0.31 ± 0.01^b	0.432 ± 0.01^c	0.27 ± 0.01^b	0.425 ± 0.01^c
S	0.816 ± 0.19^a	0.831 ± 0.015^a	0.6465 ± 0.06^a	0.3965 ± 0.08^a	0.57 ± 0.06^a

Cl	5.3225 ± 2.16[a]	7.3 ± 0.28[a]	10.125 ± 0.08[a]	5.85 ± 0.212[a]	6.76 ± 3.3[a]
K	268.29 ± 55.6[a]	393.27 ± 11.3[a]	298.55 ± 0.11[a]	426.78 ± 16.7[a]	272.6 ± 26.5[a]
Ca	123.6 ± 45.57[a]	125.1 ± 11.3[a]	79.375 ± 1.92[a]	121.975 ± 14.2[a]	85.9 ± 11.8a
Cr	0.0861 ± 0.02[a]	0.254 ± 0.008[b]	0.16875 ± 0.01[a]	0.0738 ± 0.003[a]	0.075 ± 0.02[a]
Mn	4.15 ± 1.55[a]	6.325 ± 1.73[a]	5.075 ± 0.9[a]	2.6250 ± 0.318[a]	4.45 ± 0.07[a]
Fe	43.8 ± 1.69[a]	84.4 ± 1.69[a]	52.875 ± 0.9[a]	28.5 ± 1.27[a]	25.2 ± 1.2[a]
Ni	0.542 ± 0.4[a]	0.969 ± 0.09[a]	0.56875 ± 0.01[a]	0.26575 ± 0.03[a]	0.221 ± 0.07[a]
Cu	0.6695 ± 0.49[a]	3.7 ± 0.07[b]	1.95 ± 0.176[a]	0.45175 ± 0.06[a]	1.45 ± 0.83[a]
Zn	7.55 ± 0.49[a]	12.625 ± 2.08[a]	10.45 ± 0.35[a]	8 ± 0.84[a]	10.2 ± 3.76[a]
As	ND[a]	5.75 ± 0.424[b]	0.95 ± 0.016[c]	0.670 ± 0.19[d]	5.34 ± 2.4[e]

Table 4.The mean estimated daily intake (MEDI) (mg/kg) of essential elements in CaC_2 treated mangoes. The provisional tolerable daily intakes (PTDI) recommended by Joint FAO/WHO Expert Committee on Food Additives.

	Mean Estimated Daily Intakes(MEDI)					Provisional Tolerable
Elements	**T1**	**T2**	**T3**	**T4**	**T5**	**Daily Intakes (PTDI)**
Ca	0.25	0.3	0.356	0.348	0.273	-
Cr	0.00062	0.0008	0.0005	0.002	0.002	1.5
Mn	0.013	0.210	0.016	0.008	0.014	0.14
Fe	0.146	0.281	0.176	0.095	0.084	0.7
Ni	0.001	0.003	0.001	0.0008	0.0007	0.02
Cu	0.0022	0.012	0.0065	0.0015	0.0048	0.4
Zn	0.25	0.421	0.034	0.026	0.034	0.3
As	ND	0.019	0.003	0.002	0.017	0.0003

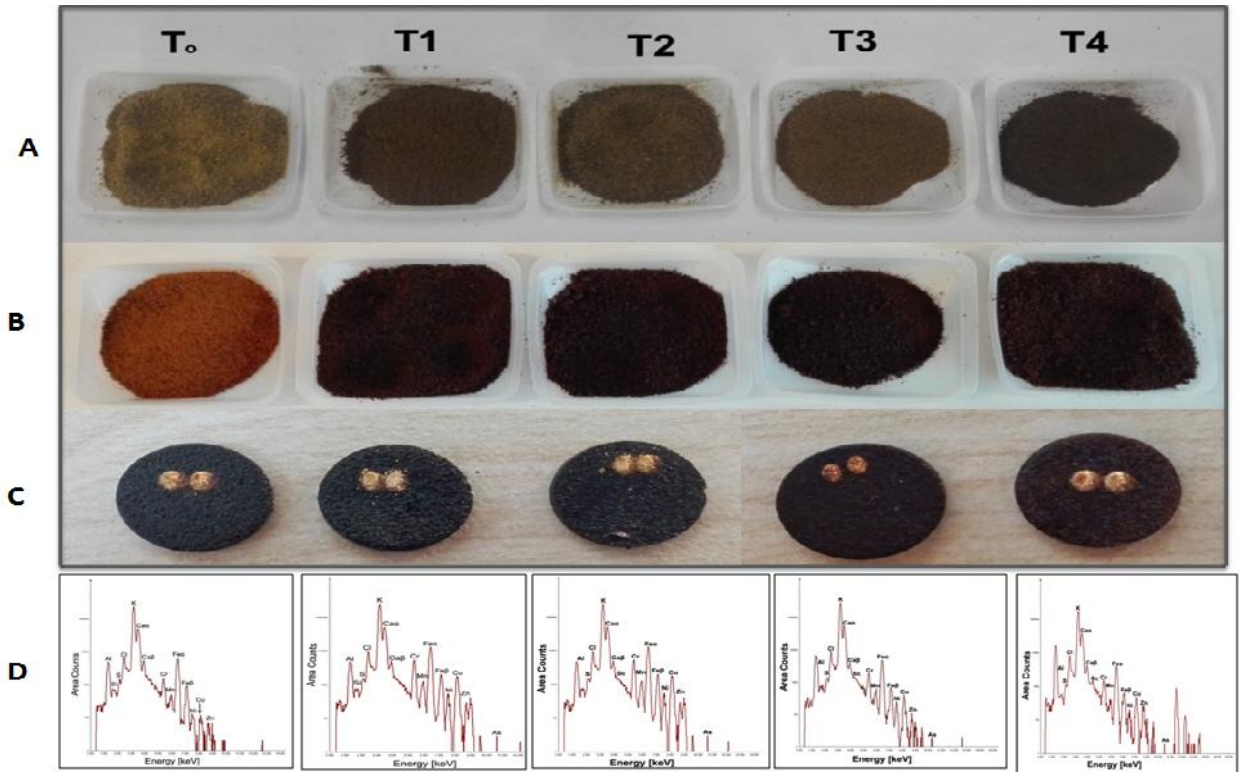

Figure 1: PIXE Analysis mango fruits with different concentrations of CaC_2 treatments in comparisons to control (non treated fruits) and mango samples from markets. Fresh unripe mango fruitsamples were divided into five different groups and treated with CaC_2 from T_0-T_4 as explained in Table-1. **A)** Ground mango fruit pulp samples demonstrating different groups of treatments**. B)** Ground mango fruit peel samples demonstrating different groups of treatments**. C)** Pallets of mango fruit pulp samples were irradiated using 3 Me V Protons for PIXE Analysis. D) PIXE spectrum of CaC_2 treated groups of Mangoes. X-axis represents the energy of element and Y-axis represents the Area counts of different metals.

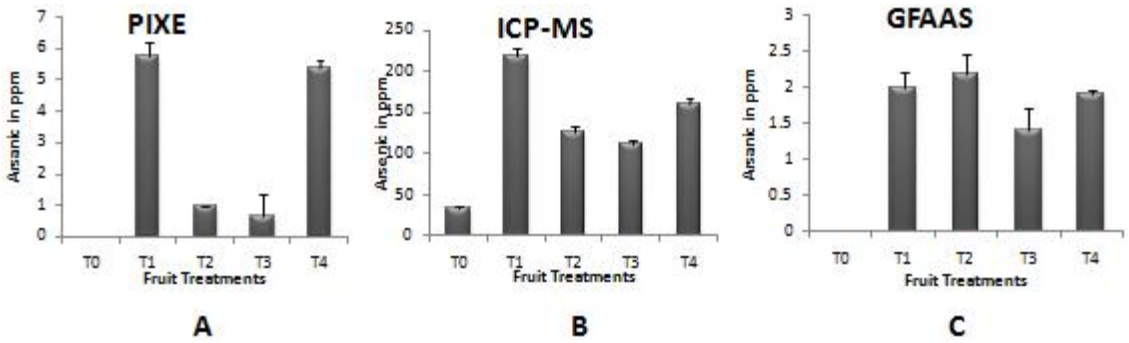

Figure 2. Analysis of mango fruits treated with different concentrations of CaC_2 and market fruit samples with PIXE, ICP-MS and GFAAS. Fresh unripe mango fruitsamples were divided into five different groups and treated with CaC_2 from T_0-T_4 as explained in Table-1. Presence of arsenic was analyzed in ground mango fruit pulp samples by three different independent methods, **A)** PIXE, **B)** ICP-MSand **C)** GFAAS.X-axis represents CaC_2 treatments and Y-axis represents presence of Arsenic in ppm.

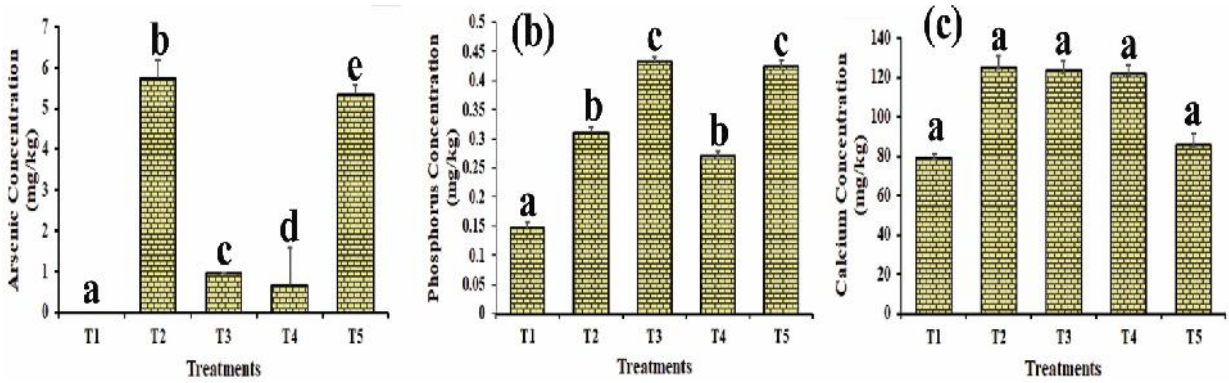

Figure 3:Effect of different treatments of calcium carbide on arsenic, phosphorus and calcium content of ripened mangoes fruit. Mature and green mango were collected from orchid and treated with different concentration of CaC_2. Artificially ripened were ground to fine powder and arsenic was quantified (mg/kg) using PIXE. In this figure **(a)** represent arsenic concentration in mangoes**(b**) represent phosphorus concentration in mangoes and **(c)** represent calcium concentration in mangoes. X-axis shows the group based on CaC_2 treatment and Y axis shows the amount of arsenic (mg/kg) in artificially ripened mangoes.One way ANOVA with post-hoc Tukey HSD test was performed(https://astatsa.com/OneWay_Anova_with_TukeyHSD/). Same letters indicate no significant difference and the error bars represent the least squaremean± SEM, n=3.

Conclusions: This work provides the first direct evidence of the source of As contamination in artificially ripened mangoes throughthe use of calcium carbide, which is routinely used in Pakistan for fruit ripening especially for local consumption. Estimated daily intake arsenic levels are more than the WHO recommended guidelines and the higher rate of mango consumption during season can accumulate dangerous levels of As in our bodies through CaC_2 treated fruits.Due to potential health risks, the use of calcium carbide is strongly discouragedworldwide and regulatory measures should be urgently taken to reduce the exposure of this carcinogenic element from the fruits like developed countries by using alternative post harvest technologies. Therefore, this hazardous chemical should be totally banned and further extensive studies are required to establish updated food safety/regulatory guidelines for Pakistani postharvest local technologies.This study will also help to educate the local fruit industry as well as the consumers to eliminate this cancer causing trace elements from our fruits.

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