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Microbial And Heavy Metals Analysis In Mango (*Mangifera indica* L.) Fruit Varieties Sold In Katsina State, Nigeria

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ABSTRACT

The use of ripening agents to trigger uniform and quick ripening of Mango has been associated with heavy metal intoxication. Determining the levels of heavy metals in Mangoes is paramount important in making our food safe for consumption. Proximate analysis of each sample of mango fruit was conducted for the content of moisture, ash, crude fiber, fat, protein and carbohydrate according to the AOAC the data was evaluated at different types of mangoes in katsina state municipal, significance test were made using the analysis of variance (ANOVA) by using Duncan multiple range test or postahoc test. Samples of ripe mango fruits of different varieties were collected from vendors of the central market Katsina and Central motor park Katsina, Katsina State. The samples were identified and the Heavy metals are detected with aid of Atomic Absorption Spectrophotometer (AAS) model A-6200, Agilent crop, Kyoto, Japan. The results obtained reveals 0.570 mg of Fe against 0.80 (WHO), 0.510 mg of Zn against 0.320(WHO), 0.431 mg of Co against 0.05 (WHO), Pb not detected, and 0.106 mg of Ni against 1.40 (WHO) among the essential metals, concentration of each Zn and Co in the mango sample were higher 0.510 and 0.431 respectively, but all other metals among measured lies within the maximum permissible limit of WHO. High concentration of the metals is due to exposure to polluted environment of production and contamination during transportation, the composition of the soil, irrigation water and storage practices application of fertilizers and pesticides and ripening agent such as Calcium Carbide Could also be the reason for it. The nutritional content of mango fruits were cited out with the characteristic features of health benefits of mango fruits and its effect. The finding recommends Periodical studies of concentration of heavy metals should be mounted on fruits and vegetables supplied to the health risk of the people the Katsina state and that government should have an agency charged with the responsibility of regulating the use of pesticides and ripening agents by mango sellers to make every food and vegetable safe for consumption especially guided by seasonal variations unregulated use of fertilizers on farms neighboring the dam should be discouraged government showed set up a regulatory body for continuous monitoring of heavy metals in fruits and vegetables.

Keywords: Heavy metals, Proximate analysis, Microbial analysis, Mango fruit and Katsina

INTRODUCTION

Fruits are vital to the human diet due to its composition of carbohydrate, minerals, vitamins and trace elements which are important for the maintenance of health, prevention and treatment of diseases (1-3). Mango (*Mangifera indica* L.) is one of the most cultivated fruit in the tropical and subtropical regions. Mango is native to South Asia, where from it has Spread worldwide to become one of the most

extensively exploited fruits (Hoque et al., 2018). It belongs to the family Anacardiaceae and is considered as the King of all fruits.

Mango is now grown commercially in more than 111 countries (Rymbai et al., 2013) with Nigeria ranking ninth among these mango-producing countries (Ugese et al., 2012) and Benue state ranking first (1st) in the league of states that produce mangos in Nigeria (Ubwa et al., 2014). The success of mango cultivation in Nigeria can be attributed to the diverse and favorable environmental conditions across the country. Mango is a valuable and popular fruit possessing rich dietary source (carbohydrates, fiber minerals), antioxidants such as vitamin C, carotenoids, and phenolic compounds which have shown various health benefits (Gámez et al., 2017; Liu et al., 2014). The increasing rate of malnutrition in Nigeria (USAID, 2017) has resulted in a growing interest in functional foods that can provide not only the basic nutritional and energy requirements but also additional physiological benefits. A functional food is known to produce a beneficial effect in one or more physiological functions and protect against disease via several mechanisms (Apak et al., 2007; Rymbai et al., 2013).

Many factors have been associated with contaminating foods with heavy metals. Some of which includes; absorption of heavy metals from contaminated water during irrigation, addition of fertilizers and metal-based pesticides, fallouts of industrial and urban emissions, harvesting process, storage and during sales (Ikechukwu et al., 2019; Abdelkareem et al., 2018). Heavy metals of nutrition concern include Arsenic (As), Mercury (Hg), Lead (Pb), and Cadmium (Cd) (Ikechukwu et al., 2019). Studies have reported that the commercial grades of calcium carbide used in artificial ripening of banana are considered hazardous as they contain traces of arsenic and phosphorus hydride which are poisonous to humans and causes health related 7 | o f 2 3 issues including cardiovascular disorders, cancers, gastrointestinal disorders and renal failure (Fattah and Ali 2010).

In Nigeria, large quantity of fruits are produced in millions of tons and estimated as annual production. Major fruits produced in Nigeria include mango, pineapple, plantain, banana, citrus, guava, pawpaw, avocados and water melon (Ibeawuchi et al., 2015). Transporting and distributing of fruits from farms to markets can take several days. During this time the naturally ripened fruits may become over ripened and spoiled. A part from spoilage naturally ripened fruits can also be damaged during transportation. These increase economic loss for the fruit sellers and therefore to minimize the loss, fruit sellers sometimes prefer harvesting fruits before full ripening and later ripen the fruits artificially before selling to the consumers (Mursalat et al., 2013). This process of maturation can be artificially accelerated by using different chemicals of which calcium carbide is the most commonly used. Investigations by Fattah and Ali (2010), revealed that many wholesalers used to sell fruits ripened by chemicals to avoid financial loss. Reports have shown that a number of chemical and biological agents are used for artificial ripening of fruits. Some of the chemical agents include ethylene gas, ethephon, ethylene glycol, etherel and carbides of Calcium and Potassium (Singal et al., 2012). Calcium carbide used to induced and accelerate ripening of fruits food is considered extremely hazardous, because it contains traces of the heavy metal arsenic and phosphorous (Anwar et al., 2008). Despite all this health implications use of chemical ripening agents is not regulated in Nigeria probably due to lack of awareness by stake holders such as policy makers, farmers, traders and consumers, food and drugs regulatory agencies. Thus, the need to investigate the extent to which these ripening agents affect the quality of the fruits and nutritional value sold in our markets.

MATERIALS AND METHODS

Sample Collection and Preparation

Samples of ripe mango fruits of different varieties will be collected from vendors of the central market Katsina and Central motor park Katsina, Katsina State. Samples will be taken to the herbarium Laboratory of Umaru Musa Yar'adua University for identification and storage for further use. All the selected samples will be fresh; undamaged, firm and healthy.

Preparation of Media

Preparation of Sarboroud Dextrose Agar (SDA)

Sarbouraud dextrose agar (SDA) was prepared according to manufacturer's instructions, SDA (65g) will be dissolved in 1000 ml distilled water and 0.5g streptomycin will be added to inhibit bacterial growth. The conical flask were plugged with cotton and capped with aluminum foil,sterilizing using lender autoclave at 121°C for 15 minutes, cooled to 45° before been poured into sterilized plates and kept at 30°C (Cheesebrough, 2009)

Preparation of Potato Dextrose Agar (PDA)

Potato dextrose agar (PDA) will be prepared according to manufacturer's instructions, 39g PDA will be dissolved in 1000 ml of distilled water, the suspension will be mixed until completely homogenized and 0.5g of streptomycin was added to inhibit bacterial growth. The conical flask containing the media were plugged with cotton wool and capped with aluminum foil, sterilized using lender autoclave at 121°C for 15 minutes, cooled for 45°C and pouring in to sterile plates. The plates will be kept at 30° (Cheesebrough, 2009).

Preparation of Nutrient Agar (NA)

Nutrient agar (NA) was prepared according to manufactures instructions; 28g NA was dissolved in 1000ml of distilled water, the suspension was mixed until completely homogenized. The conical flask containing the media was plugged with cotton wool and capped with aluminum foil. The flask was sterilized using lender autoclave at 121°C for 15minutes, cooled to 30°C and poured into sterile plates Biotech Laboratories ltd (Cheesebrough, 2009).

Sample preparation for wet digestion

For estimation of arsenic and calcium on the fruit surface, 20g of fruit from each treatment was washed in 1 litre of double distilled water and was diped for 30 min. The washing water was used for estimation of residues on the fruit surface. For peel and pulp, 1kg each of peel and pulp was crushed and homogenized in a blender separately.

Wet digestion method

Twenty grams (20g) of homogenized sample (pulp and peel) and 20cm³ of washing water was placed into the digestion flask. A few glass beads, 10ml of H₂SO₄ and 10ml HNO₃ has been added in the digestion flask. The mixture in digestion flask was heated gently until the liquid appreciably darkened in colour. Then, HNO₃ was added in small proportions (1 to 2ml) and heating continued until darkening again took place. Further, nitric acid is added continuously and heated to fuming for 5 to 10 mint until the solution becomes clear. When all the organic matter is oxidized, the solution were allowed to cool. To this solution, 10 ml of distilled water was added and whole mixture is boiling gently to fuming. On cooling, 5 ml water is added to the solution and again boiled gently to fuming. Finally, the digest cooled filtered in to 100cm³ volumetric flask and then made up to the mark using double distilled water, then, the residues of arsenic in different aliquots was determined by using ICP-AES (Inductively Coupled Argon Plasma-Atomic Emission Spectrometry) while Calcium was determined by using Flame Photometric method.

Microbial Analysis

Enumeration of Microbial Load

A ten-fold serial dilution of each of the samples was carried out. Spread plate technique was employed by inoculating 0.1ml aliquot aseptically from the 10⁻³ and 10⁻⁴ dilutions onto nutrient and MacConkey agar plates for enumeration of bacteria and Sabouraud dextrose agar for fungi count. The agar plates was incubated at 37°C for 24-48 hours for bacterial count and at 25°C-28°C for 5-7 days for fungal count. Each sample was inoculated in duplicate agar plates and the mean values of bacterial and fungal counts was recorded as colony forming unit per ml (cfu/ml) Obunukwu et al. (2018).

Isolation of Fungi

A total of thirty (30) randomly selected samples of spoiled fruits of mango of different varieties and another thirty (30) healthy looking fruits of different varieties, Five (5) of each variety was examined for microbial spoilage. Fruits was cut into small segments (3mm in diameter) with a sterilized blade, surface

sterilized in 1% hypochlorite for 2 min, plated on Sabouraud dextrose agar (SDA) aseptically and then incubated at 28°C for 5 days (Mailafia et al., 2017).

Purification of Fungal Isolates

Colonies from the primary plates was aseptically picked with a sterile inoculation needle and transferred onto a freshly prepared sterile Sabouraud Dextrose Agar (SDA) plate with a streaking method and incubated for 5-7 days at 25°C-28°C. Discrete colonies was aseptically transferred and stocked on slant and incubated for another 5 days at 25°C-28°C. Pure colonies was stored in the refrigerator at 10°C-15°C until needed for characterization and identification Obunukwu et al. (2018).

Identification of Isolated Fungi and Bacteria

Fungal Identification

Fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation. The technique of Oyeleke and Manga (2008) was adopted for the identification of the isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was mounted and viewed under the light microscope with x40 objective lenses. The morphological characteristics and appearance of the fungal organisms seen was identified in accordance with Adebayo-Tayo et al. (2012), Onuorah et al., (2015) Klich (2002), Samson and Varga (2007).

Isolation of Bacteria

Bacterial population of the mango fruit samples was enumerated by serially diluting 1g of spoiled mango fruits. From the seventh dilution, (10⁻⁷) 1ml was transferred into sterile molten nutrient agar plates using a dropper pipette, spreaded using bend glass rod. The plates were incubated at 30°C for 24 hrs (Chikere et al., 2009). The number of viable bacteria, in the samples was estimated from the number of colonies formed using a colony counters and expressed as CFU/g Obunukwu et al. (2018).

Purification of Bacterial Isolates

Colonies from the primary plates was aseptically picked with a sterile wire loop and transferred onto freshly prepared sterile nutrient agar plate, with a streaking technique such that discrete colonies appear at the ends of streaked lines after incubation. The subculture plates was incubated at 37°C for 24 hours. Discrete colonies from the subculture plates was aseptically transferred and streaked on slant and incubated for another 24 hours at 37°C which was stored at 4°C and used subsequently for microscopic characterization and biochemical analyses Obunukwu et al. (2018).

Characterization and Identification of Bacterial Isolates

All bacterial isolates will be characterized and identified based on their cultural, morphological, microscopic examination and biochemical characteristics following the methods prescribed by (Cheesbrough, 2005). Biochemical test conducted include the following: Gram stain, Catalase test, Oxidase test, Motility test, Methyl red test, Citrate test and Urease test Obunukwu et al. (2018).

Proximate composition and Minerals analysis

Proximate Analysis

Naturally ripe Mango fruits was taken to Agricultural Chemical laboratory of Federal University Dutsin-Ma for the determination of proximate composition which includes determination of moisture, ash, lipid, protein, fibre and carbohydrates contents of the fruits as described by AOAC (2002).

Determination of heavy metals

30 samples of mangoes where from 3 different markets in katsina state municipal washed with distilled water and dried, the pulp of a mango was separated from the kernel and stored in a refrigerator below 10°C, extracted mango pulp mango was dried in oven at 120°C for 6hrs until it becomes crispy and brittle, the solid mass was grinded by the use of pestle and motor, which was washed with acid. Exact 5.0g of each of the grinded powder sample was heated in silica crucible in muffle furnace at 250°C for 6 hours.

Heated samples were taken out, cooled and treated with 2.00ml of concentrated HNO₃ and covered with watch glass, it was further heated at a temperature of 60^oc for 2hrs and allow for cooling, 5ml of 8.25 HCL was added to dissolve the sample and digested using the hot plate, the digested one was cooled. 15ml of distilled water added and filtered using filter paper. The filtrate obtain was diluted up to 40ml by the addition of distilled water.

RESULTS AND DICUSSIONS

Proximate analysis of the Sample (Mango Fruits):

90% moisture, 8.8% carbohydrate, 0.07% protein, 0.1 calcium, 4.5mg/100g, Fe 6.3-20.2mg/100g carotene as vitamin A30mg/100g ribophorin and 3mg/100g ascorbic acid (Bose and Mila 2001).

Mango fruits also contain structure structural carbohydrate such as pectin and cellulose, the major amino acid include lysine, leucine, cysteine, valine, arginine, phenylamine and methionine. (Bose & milla 2001)

Mineral Composition:

- Potassium (K)
- Calcium (Ca)
- Magnesium (Mg)
- The content of iron, sodium, and Zinc varies depending on the mango varieties

Proximate Analysis: the proximate analysis of each sample of mango fruit was conducted for the content of moisture, ash, crude fiber, fat, protein and carbohydrate according to the AOAC the data was evaluated at different types of mangoes in katsina state municipal, significance test were made using the analysis of variance (ANOVA) by using Duncan multiple range test or postahoc test.

Table 1. Shows the weight and volume of different types of mango fruit. The the higher value are for fruits with higher moisture contents was for Kmpmf and Kymf (78.52 ± 0.01 and 78.7 ± 0.0028) and the lower content is for Kcmmf ($77.4 \pm 0.01\%$) the data are within the range of (74.58-86.36 as reported by Joseph & Aworth. The crude protein content of Kmpmf and Kymf (0.74 ± 0.002 and 0.79 ± 0.018) respectively are lower than the content of Kcmmf ($0.82 \pm 0.03\%$) mango with higher content of ash is far kcmmf ($1.7 \pm 0.02\%$) and the lower values was for the Kmpmf and Kymf (1.5 ± 0.02) and ($1.35 \pm 0.01\%$) the crude but for the content Kcmmf (0.38 ± 0.02 and $0.31 \pm 0.1\%$) no variable in crude fiber contentents among Kcmmf, Kmpmf (4.5 ± 0.01 , 4.2 ± 0.01 and $4.4 \pm 0.03\%$) fruit mango, fruits with higher contents of carbohydrate are for Kcmmf and Kmpmf (15.4 ± 0.01) and $14.9 \pm 0.3\%$) while the lower content is for the Kcmmf (14.1 ± 0.01).

Table 1: Proximate Analysis of Different types of Mango Sample Treatment.

Composition (%)	KCMMF	KMPMF	KYMF
Moisture	77.40 ± 0.01	73.52 ± 0.01	78.70 ± 0.02
Ash	1.50 ± 0.02	1.35 ± 0.01	1.70 ± 0.02
Crude Protein	0.82 ± 0.03	0.74 ± 0.02	0.79 ± 0.03
Crude Fiber	4.50 ± 0.01	4.20 ± 0.01	4.40 ± 0.03
Fat	0.38 ± 0.01	0.29 ± 0.02	0.31 ± 0.01
Carbohydrate	15.40 ± 0.01	14.90 ± 0.03	14.10 ± 0.1

It's concluded that the KCMMF had a good quality but there is significance difference as regards to its appearance, color, flavor content and their overall acceptability.

Determination of the Heavy metals

The digested samples were analyzed for heavy metals (Fe, Zn, Co, Pb and NL) by spectrometric determination, heavy metals concentrations in the mango fruit were analyzed with the maximum limit of WHO the mean values and SD of concentration was determined.

IRON: Iron is one of the abundant component of human blood. The deficiency could result in Anemia from the results showed that the concentration of iron in mango fruits is 0.540 ± 0.48 mg/kg

ZINC: Zinc is the major component of many Co-enzymes essentials for the building up of Protein DNA, RNA, and insulin the concentration of Zinc in the mango fruits is 0.510 ± 0.031 mg/kg which is higher than permissible limit of WHO (0.30) mg/kg

COBALT: Cobalt is the integral part of Vit B-12 it enhance in the production of red blood cells. Anti-Viral and anti-bacterial compounds high intake of cobalt resulted in the high production of red blood cells. The concentration of cobalt found in mango fruit is 0.431 ± 0.021 mg/kg higher than the WHO permissible limit.

LEAD: Lead usually enter the body through air, water and food, and it's very dangerous to human health.

NIKEL: Plays an important role in the production of insulin its deficiency can easily affects liver, the concentration of Nickel found is 0.106 ± 0.003 mg/kg which is below the limit of (Ubwa, S.T. *et al.*, 2014

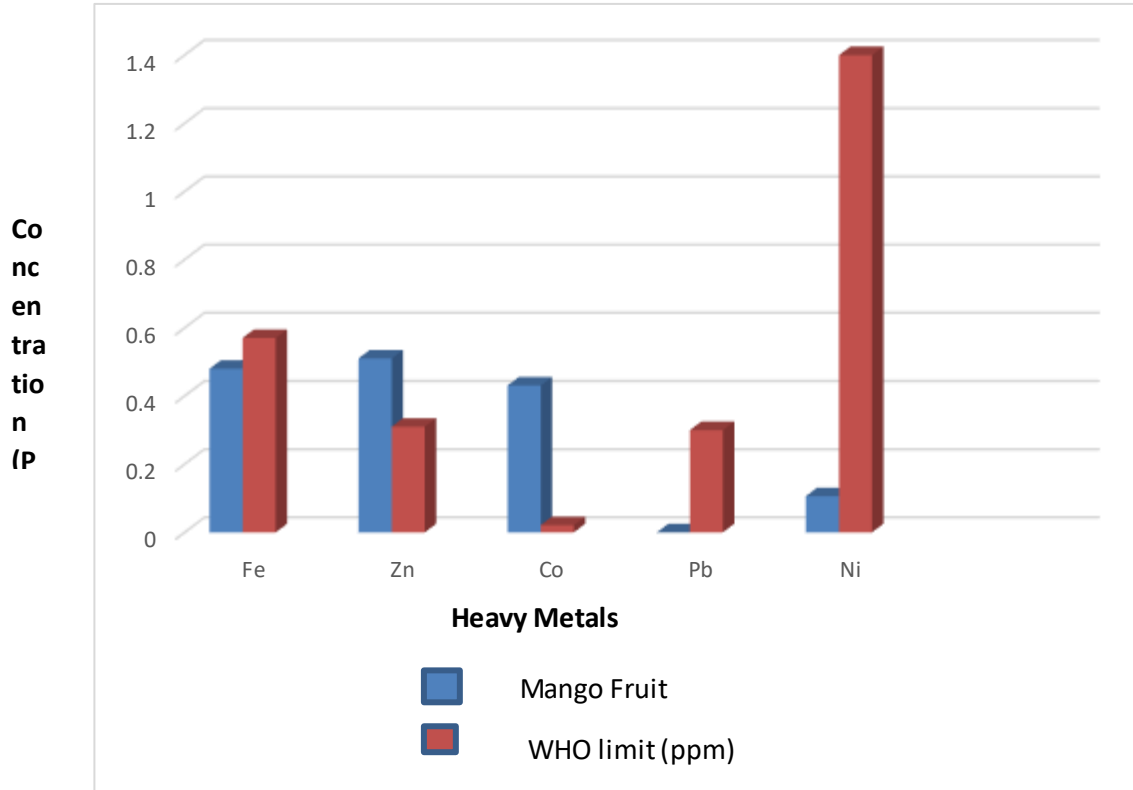
Among the metals analysed the concentration of Zn and Cobalt were found higher than the maximum limits of WHO whereas the concentration of Iron lead and nickel were within the acceptability limit. The higher concentration of the metals maybe due to source of production as it's comes from different local government of the state. There are contamination during transportation, exposure to the environmental pollution, pollutants found in irrigation water, soil, application of fertilizers, pesticides and application of Calcium Carbide for quick and easy ripening of the fruits could be responsible for the accumulation of these metals.

The high concentration of cobalt is due to exposure of production to industrial wastage dumpsite containing decomposed batteries.

Table 2: Shows the concentration of heavy metals in mango fruits in part per million ppm and reported as mg/kg the comparative bar Diagram of Fe, Zn, Co, Pb, and Ni for mango fruits.

Average Concentration of Heavy metals (means n=15, mg/kg or ppm dry weight in mango)

Heavy Metals	Mango Samples	Permissible Concentration by WHO (ppm)
Fe	0.570 ± 0.48	0.80
Zn	0.510 ± 0.031	0.320
Co	0.431 ± 0.021	0.05
Pb	-	0.3
Ni	0.106 ± 0.003	1.40



Graphical Representation Concentration of Fe, Zn, Co, Pb and Ni in Mango Samples

The results obtained reveals that among the essential metals, concentration of each Zn and Co in the mango sample were higher but all other metals among measured lies within the maximum permissible limit of WHO. High concentration of the metals is due to exposure to polluted environment of production and contamination during transportation, the composition of the soil, irrigation water and storage practices application of fertilizers and pesticides and opening agent such as Calcium Carbide Could also be the reason for it. The results indicate that high consumption of Zn and Cobalt shows high amount of Zn and Co. Regular monitoring of heavy metals in fruits and vegetables supplied to the health risk of the people the Katsina state government showed set up a regulatory body for continuous monitoring of heavy metals in fruits and vegetables.

Table 3: Shows the Nutritional contents of mango amount per 100gms.

Calories	%Daily Value
- Fat 0.4g	0%
- Saturated Fat 0.1g	0%
- Cholestrol 0.mg	0%
- Sodium 1 mg	0%
- Potassium 168g	4%
- Carbohydrate 15g	5%
- Dietyry fibre 1.6g	6%
- Sugar 14g	-
- Protein 0.8g	1%
- Vitamin C 60%	1%
- Iron 1%	-
- Vitamin B 5%	-
- Magnesium 2%	-
- Calcium 1%	-
- Vitamin D 0%	-
- Cobalamin 0%	-

Source: National institute of health NIM <https://pmc.ncbi.nlm.nih.gov>. (gizachew girma etal 2016).

Health Benefit of Mango are as follows:

- Control blood pressure
- Skin Cleanser
- Improve eye sight
- Boost immunity and brain health
- Anti-agent benefits
- Reduce Cholesterol level
- Prevent Diabetes
- Improve Digestion
- Promote weight loss
- Promote hair grow
- Aphrodisiac qualities
- Gives relief from Anemia
- It helps in pregnancy
- Elimination of Egestion and acidity
- Helps to maintain healthy heart
- It cures acne and aging process (Fatah & Ali, 2010).

CONCLUSION AND RECOMMENDATION

Conclusively the proximate chemical composition of verities of mango were analysed accompanied with their parameters such moisture, ash, protein, crude fibre, fat and carbohydrates with their different percentage composition demonstrated significantly enough on its analysis. Heavy metals are detected with aid of spectrometric analysis such as Fe, Zn, Co, Ni, Pb Comparatively and chemically the concentration of Zn, and Co is higher than Ni and Pb. The nutritional content of mango fruits were cited out with the characteristic features of health benefits of mango fruits and its effect.

Therefore it's recommended for national and international regulations on food and vegetable. Safety set the optimum levels of heavy metals in food fruits and vegetables. Regular supplied to the markets is essential to reduce health risk of the people. The government should set up a regulatory body for continuous monitoring of heavy metals in fruits and vegetable. Government should make a schedule for monitoring storage of mango before dispatch to the markets. Most of the vendors applied calcium carbide for easy ripening it result in Cardio Vascular diseases and cancer. The Government should guides relevant authorities to regulate the use of pesticides and ripening agents used by mango sellers to make every food and vegetables safe for consumption. The Government should need to investigate the extent to which these ripening agents affects the quality of the fruits and Nutritional value sold in our markets. Research institute should always monitor the consumption of good, fruits and vegetable. All institutions such as university, college of education polytechnics should always make researches on any food, fruits and vegetable consumes becomes harmful to living system.

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